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THOMPSON YATES AND JOHNSTON LABORATORIES REPORT

THE THOMPSON YATES AND JOHNSTON LABORATORIES REPORT

EDITED BY
RUBERT BOYCE AND CHARLES S. SHERRINGTON
WITH
H. E. ANNETT, BENJAMIN MOORE, RONALD ROSS
AND E. W. HOPE

ASSISTED BY
W. B. WARRINGTON, E. E. GLYNN
J. W. W. STEPHENS, A. E. SHIPLEY, J. E. DUTTON, J. L. TODD
H. WOLFERSTAN THOMAS, H. E. ROAF, CUTHBERT CHRISTY
S. F. LINTON, AND E. S. EDIE

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OF THE
LIVERPOOL SCHOOL OF TROPICAL MEDICINE
AND MEDICAL PARASITOLOGY

BY
J. EVERETT DUTTON, M.B., Vict.

JOHN L. TODD, M.D., McGill
AND
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BY
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AND A NOTE ON TSETSE-FLIES

BY
E. E. AUSTEN
ZOOLOGICAL DEPARTMENT, BRITISH MUSEUM

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AUGUST, 1904

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PREFACE

IN 1901 trypanosomes were discovered in the blood of a European by Dr. J. E. DUTTON, Walter Myers Fellow, while on an Expedition of the Liverpool School of Tropical Medicine to Gambia. In consequence of this observation an Expedition composed of Drs. DUTTON and TODD was sent in 1902 by the School to Senegambia to prosecute further researches in trypanosomiasis. The detailed report of the Expedition was published in 1903, and contained a study of the pathogenic trypanosomata of man and animals, several new species being described.

Prior to the return of this Expedition, the discovery of trypanosomes in the cerebro-spinal fluid of cases of Sleeping Sickness in Uganda by members of the Sleeping Sickness Commission of the Royal Society caused the subject of trypanosomiasis to assume great importance. At the same time it was brought to the notice of the Committee of the Liverpool School that in the Congo Free State the native population had from time to time suffered from very fatal epidemics of this disease. The Committee therefore decided to accept the invitation of His Majesty King LEOPOLD to send an Expedition to study Sleeping Sickness in that country. Drs. DUTTON and TODD were recalled from the Senegambia, and as soon as they had drawn up their reports they left for the Congo in September, 1903, and were soon after joined by Dr. CHRISTY, who had served previously on the Royal Society's Sleeping Sickness Commission in Uganda. On reaching the Congo the Expedition decided to make Leopoldville its headquarters. The authorities of the Free State at the same time attached Dr. INGE HEIBERG, an old pupil of the School, to the Expedition, and to him the members are greatly indebted for his aid in the work. A special hospital was erected by the State, in order that the observers might have the Sleeping Sickness cases under their care, and facilities were given for the study of a large number of patients. The results of these investigators are incorporated in the present volume, and illustrate the occurrence and distribution; describe the symptoms of trypanosomiasis in all its stages, both in Europeans and natives, and shew how Sleeping Sickness, so-called, is related to trypanosomiasis as a symptom of that disease.

At the same time the Committee resolved to continue the researches on trypanosomiasis in Liverpool, which had been started by Drs. DUTTON and TODD in Senegambia ; Dr. THOMAS was appointed to conduct the work, and aided by Dr. LINTON experiments were immediately commenced, a preliminary note of their work being embodied in this report. The two groups of observers have throughout worked together, and in order that comparable data might be obtained, selected cases of Sleeping Sickness were, by permission of the Congo Free State authorities, sent to the observers in Liverpool. A later report will be published on these cases. As far as the very numerous and detailed observations of these workers go, they shew that the parasite identified with Sleeping Sickness in Uganda and the Congo does not differ from that described by DUTTON in the Gambia. This view is also held by LAVERAN and MESNIL in France, and BRUCE in this country. The question of a curative agent has for a considerable time engaged the attention of the members of the research, and experiments are now in progress to find a remedial agent which would have the same effect in trypanosomiasis that quinine has in malaria. A variety of drugs have been used with more or less success ; up-to-date, arsenic and trypan red, an aniline dye introduced by EHRLICH and SHIGA, appear to be the most useful ; the parasite disappears for a time from the blood, and the life of the animal is prolonged, but with neither of the drugs is an absolute cure attained. A combination of the two appears to offer better results ; a large number of animals infected with different trypanosomes are under treatment. The present report also embodies an important note on the Tsetse-flies, by Mr. E. E. AUSTEN, to whom the School is much indebted for describing and identifying the Diptera obtained during the Expedition.

Much important work remains to be done ; a further study of the disease from a clinical aspect, extended experiments on the transmission of trypanosomic diseases by biting flies, and researches on the lines of SCHAUDINN's work, together with therapeutical observations in patients and large animals naturally infected with trypanosomes, are urgently needed.

CONTENTS

HUMAN TRYPANOSOMIASIS ON THE CONGO

HUMAN TRYPANOSOMIASIS ON THE CONGO

(Being the First Progress Report of the Expedition of the Liverpool School of Tropical Medicine to the Congo, 1903) *

BY

J. EVERETT DUTTON, M.B., VICT.

WALTER MYERS FELLOW, UNIVERSITY OF LIVERPOOL

J. L. TODD, B.A., M.D., C.M., McGILL

AND

CUTHBERT CHRISTY, M.B., EDIN.

AT the request of His Majesty King Leopold II, King of the Belgians, this expedition was sent in September last to the Congo Free State to report upon the sanitation of the larger towns, and to continue the School's work on human trypanosomiasis. Through the kindness of the Governor-General of the Congo, and of Drs. VOURLOUD and NEILSENG, government physicians at Boma, the hospital for natives at Boma was opened to us. It contained about sixty-five patients, and we saw there eighteen patients who had been admitted as cases of sleeping sickness.

Whether admitted to hospital by the medical officers, or pointed out to us by the missionaries as cases of sleeping sickness, with three or four exceptions, the cases which we have as yet seen have been, in our opinion, very unlike what has hitherto been described as sleeping sickness. Continued sleep or even abnormal sleep has been almost absent from many of the cases. It has been absent even in those who were believed to be in an advanced stage of the disease and who ultimately died. In only three or four of the cases observed by us has somnolence been a marked feature, and only in these few cases have the symptoms in any way coincided with those observed by one of us during the Uganda epidemic of sleeping sickness.[1]

From November 4 to November 29 two of us were occupied in travelling through the cataract region, in order to ascertain for ourselves the exact conditions existing there. Reports sent to the Congo Free State officials, and handed on to us, as well as correspondence received by ourselves, led us to believe that in this district we should find, not only an epidemic of sleeping sickness, but that the whole population was being 'decimated' by the disease. Although, in the course of our

* *Reprinted by permission of the publishers of the* BRITISH MEDICAL JOURNAL, *January* 23, 1904

journey, we visited many villages and made tiresome expeditions to visit those especially mentioned to us, not a single case of illness did we see in which sleep was a marked feature.

We stayed for some time at the Baptist Missionary Society's station at Wathen, in the Lutete district, where we received hospitality and assistance at the hands of those in charge of the mission. From here excursions were made into the surrounding district, and a large number of persons brought to us as cases of sleeping sickness were examined.

These consisted of a heterogeneous collection of men, women, and children, many of whom were found to be suffering from heart, lung, and other more or less common ailments. Amongst the boys it seemed that a diagnosis of 'worms' was sufficient in many cases to account for the symptoms. Cases of apparent starvation and neglect were also common, and it appeared from what we were told that, owing to there being a general belief in the contagiousness of 'manimba'—the native name for what is believed to be sleeping sickness—children and even adults were, as soon as the slightest symptoms developed, liable to be isolated and shunned by everyone, causing, eventually, a state of emaciation and filth which ended sooner or later in death. Apart from these, however, and eliminating the many common ailments, there still remains in the cataract region a class of cases which, undoubtedly, terminate fatally within a year or two. These cases, of which most villages visited by us contained one to three examples, have few very evident symptoms of illness beyond emaciation, and, in some cases, weakness, headache, enlargement of lymphatic glands, and dirty, dry, scurvy skin. In a proportion of these cases trypanosomes were found in the peripheral blood, and we think it probable that if a systematic examination were possible, the parasites would be found in a much larger number.

Trypanosomes have been found in the finger blood both of those cases in which the diagnosis of sleeping sickness was certain, and of those in which the case picture was atypical. In addition, trypanosomes have been frequently seen in the peripheral blood of apparently healthy individuals. The routine method adopted for the detection of the parasites in the peripheral blood of unsuspected cases was the simple examination of a rather thick, freshly-made, cover-slip preparation. All of the following persons were examined in this way :—

Place and Class of Native	Number Examined	Number infected with Trypanosomes in Peripheral Blood	Number of Cases Admitted to Hospital or shown to us as Cases of Sleeping Sickness	
			With Trypanosomes	Without Trypanosomes
Boma				
1. Hospital at Boma. Native soldiers coming from all parts of the Congo	72	11	9 *	7
2. Prisoners in Boma gaol coming from all parts of the Congo	181	17	0	0
3. Children of native soldiers at Boma	19	0	0	0
4. Colony school. Native boys from all parts of the Congo	50	1	0	0
5. Native labourers and children from native quarter	34	0	0	0
Matadi				
1. Natives collected for examination by Dr. Sims	78	1	0	0
2. Children of native soldiers up to 8 years of age	10	0	0	0
3. Patients at the native hospital	22	3	2	2
4. Children at the Sansel, native quarters, up to 10 years of age	28	0	0	0
Cataract Region				
1. Carriers collected from Tumbar District ...	20	2	0	0
2. Carriers from Lutete District	23	0	0	0
3. Boys at Wathen Mission, ages up to 15 ...	35	1	0†	0
4. Natives examined indiscriminately at two small villages near Lutete	42	2	0	2
5. Natives coming to the mission for treatment, or sick natives seen in village within a twenty mile radius of Lutete	79	11	10	47
6. Cases collected for us as sleeping sickness cases by Chef de Post at Kusu from neighbouring villages	14	0	0	14

* Lumbar puncture was done in six of the sleeping sickness cases. In four trypanosomes were seen in the cerebro-spinal fluid. One of the four never showed trypanosomes in the peripheral blood.

† This boy had been suffering for twenty-four days previous to our arrival at the mission from a fever which was not amenable to quinine.

Case VI

Sleeping Sickness (September 30, 1903).—N'Bela, male, aged 24, agricultural labourer. A Mongo man. Patient never saw sleeping sickness before coming to Boma. Had lived in Boma for nearly three years. Illness commenced in July of this year. Admitted to hospital, August 1, 1903.

When the patient was seen (September 30) somnolence was already a marked feature of his condition. This symptom steadily became more marked as emaciation increased. When we left Boma (October 27) the patient was comatose, and death had been expected at any time during the preceding week.

General Condition.—Patient is thin and very weak. Questions are answered rationally, but only after a long interval. He displays a somnolent indifference to everything about him, and only by constant shaking can drowsiness be dispelled for a period long enough to permit him to speak.

Physical Examination.—Nervous system ; co-ordination imperfect. Sense of weight good. Knee-jerks and superficial reflexes were obtainable, and showed no abnormality. Eyes reacted to light and to accommodation. Thoracic and abdominal examination showed nothing worthy of note. Lymphatic glands were all enlarged, hard, and freely movable. There was no haemorrhage into mucous membranes. Slight icterus of the conjunctiva was seen.

The accompanying chart indicates the course of the temperature in this case, and shows the results of the examinations for parasites. Trypanosomes were seen in both cerebro-spinal fluid and peripheral blood.

On October 9, 35 c.cm. of slightly clouded, colourless cerebro-spinal fluid were taken by lumbar puncture. No red cells were seen in the fairly profuse deposit formed by centrifuging. This precipitate contained a fair number of trypanosomes, many monuclear cells of large size, and numerous polymorphonuclear leucocytes.

Experimental Inoculation

The following animals have been inoculated from this case. The material inoculated was in each case demonstrated to contain living trypanosomes.

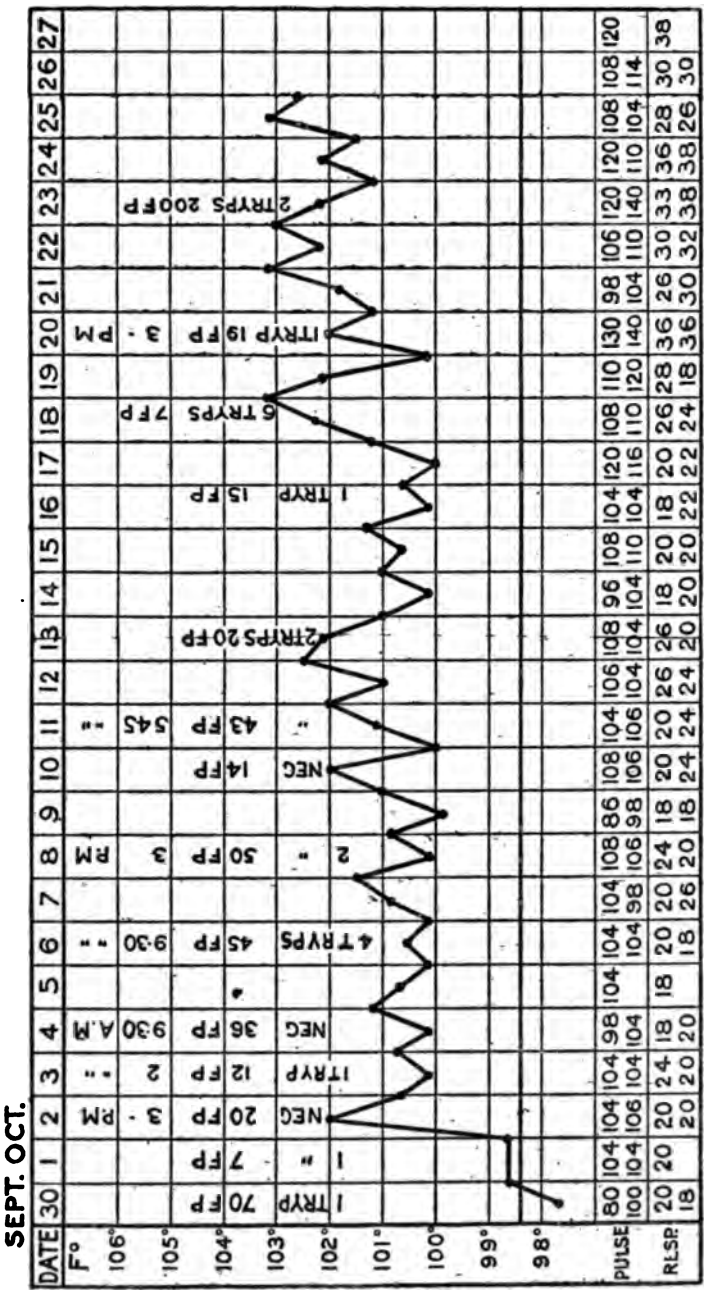
Animals Inoculated from Case 6

October 7. White mouse (Experiment 16) inoculated subcutaneously with 1 c.cm. cerebro-spinal fluid ; infected October 25.

October 7. White mouse (Experiment 17) inoculated subcutaneously with 1 c.cm. cerebro-spinal fluid ; never infected.

September 30. White rat (Experiment 5) inoculated subcutaneously with 5 c.cm. blood ; never infected.

September 30. White rat (Experiment 6) inoculated intraperitoneally with 1 c.cm. blood ; never infected.



TEMPERATURE CHART, CASE VI

F. P. = Filaria perstans

Tryp. = Trypanosome.

October 9. White rat (Experiment 21) inoculated intraperitoneally with 4 c.cm. blood ; never infected.

October 9. White rat (Experiment 22) inoculated subcutaneously with 8 c.cm. blood ; infected October 23.

October 9. White rat (Experiment 23) inoculated intraperitoneally with 5 c.cm. blood ; infected October 23.

Case IV

Simple Trypanosomiasis (' *Maladie de Dutton* ')[2], September 22.—J. P., male, aged twenty-eight, native of Sierra Leone, where sleeping sickness is not endemic. Came to Boma six years ago as a Free State soldier. Has always been in lower river districts. Entered hospital September 22 with gunshot wound. Had gonorrhoea in 1902, otherwise has not been ill during his stay in the Congo.

Patient is a strong, healthy man, well nourished, skin moist and clean ; slight oedema over both shins ; patient does not complain of feeling ill.

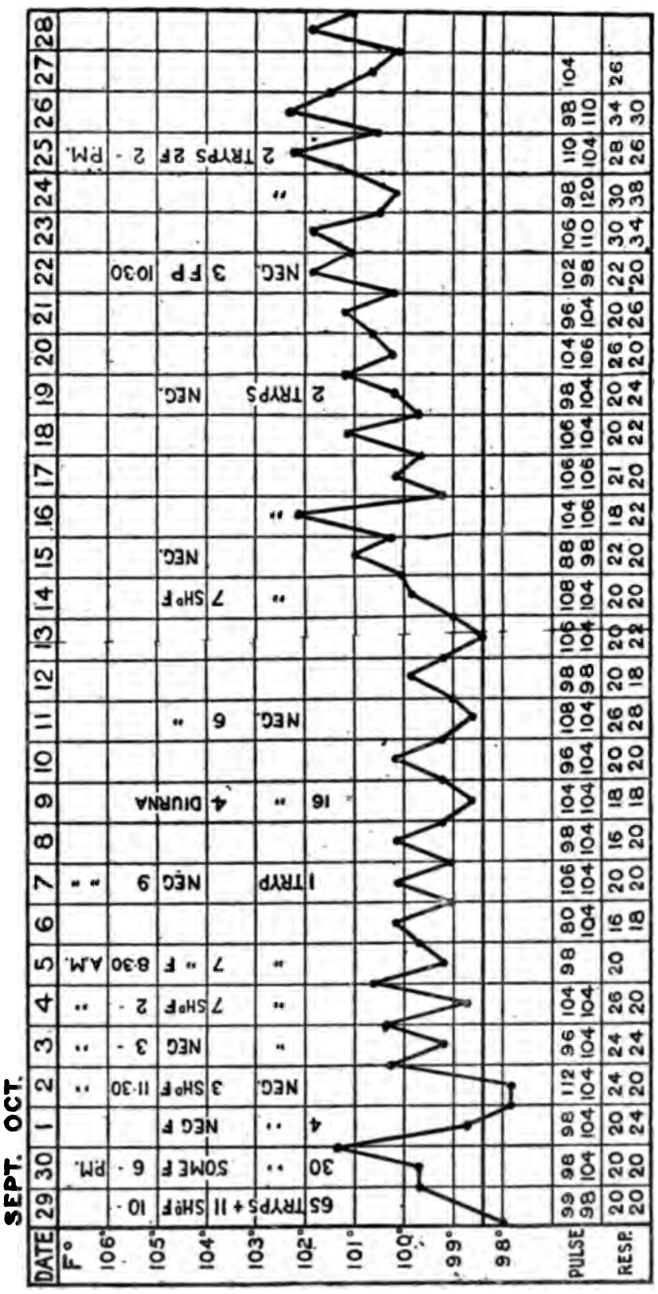
Glands are easily palpable, but are not markedly enlarged or hard. He is a bright, intelligent man, slightly deaf, but answers questions quickly and well. He is alert and interested in his surroundings. Mucous membranes are anaemic. There is a complete cataract of right eye. Heart and lungs are normal. Liver normal in size, but slightly tender. Spleen normal. Appetite good. Bowels constipated. Nervous system is normal.

Urine passed in twenty-four hours, 760 c.cm. ; specific gravity, 1,002, light straw colour, cloudy precipitate, acid ; small amount of albumen present, no sugar ; urea, 0·41 gram to 100 c.cm. of urine. Microscopically a few pus cells, probably due to a chronic gonorrhoea, were seen.

On October 26, 10 c.cm. of limpid cerebro-spinal fluid, as clear as distilled water, were withdrawn with some difficulty by lumbar puncture. The patient almost fainted before 8 c.cm. had been withdrawn. A very slight percipitate was obtained after long centrifuging. On examination it was found to contain a very few red cells, and still fewer small mononuclear white cells. No trypanosomes were seen during a long and careful search of the whole of the precipitate.

The cerebro-spinal fluid from this case presented a very different appearance, both macroscopically and microscopically, from that obtained from sleeping sickness cases. The fluid was clear, not clouded. None of the large mononuclear or smaller mononuclear and polymorphonuclear leucocytes seen in sleeping sickness cases were present.

The accompanying chart shows the course of the temperature in this case, and indicates the occasions on which trypanosomes were seen in the peripheral blood.



TEMPERATURE CHART, CASE IV

Tryp. = Trypanosome. F. P. = Filaria perstans. Shd. F. = Sheathed filaria.

Experimental Inoculations

The following animals were inoculated from this case :—

Animals Inoculated from Case 4

September 30. White rat (Experiment 5) inoculated subcutaneously with 5 c.cm. blood ; never infected.

September 30. White rat (Experiment 4) inoculated subcutaneously with 1 c.cm. blood ; infected October 8.

October 27. Guinea-pig (Experiment 27) inoculated subcutaneously with 4 c.cm. blood, infected November 7.

October 27. Guinea-pig (Experiment 28) inoculated subcutaneously with 5 c.cm. blood ; infected November 18.

October 27. Rabbit (Experiment 24) inoculated subcutaneously with 6 c.cm. blood ; never infected.

Necropsies were done at Boma on four cases which were admitted to the hospital for sleeping sickness. Two of these died before their blood or cerebro-spinal fluid could be examined. Repeated examinations of the blood (centrifuge used) of the third failed to demonstrate trypanosomes. Lumbar puncture was not done on this case. In spite of repeated and very careful examinations of the blood and cerebro-spinal fluid of the fourth case, trypanosomes were never seen.

A necropsy was also done on the body of a native admitted to the hospital as a case of encephalitis. This patient never showed the usual signs of sleeping sickness, and finally died of dysentry. Many trypanosomes were seen in his finger blood. Lumbar puncture was not done.

The *post-mortem* appearances in each of these cases were very similar to those described as occurring in sleeping sickness.[3] The usual increase of subarachnoid fluid, which had become cloudy, or occasionally almost purulent, was observed. The superficial and substantial vessels of the brain and spinal cord were turgid, and in two cases small sub-ependymal haemorrhages were noted.

In addition to these changes in each case, lymphatic glands were enlarged, several were congested or injected, not infrequently members of the various groups of glands were to the eye either partially or—especially the smallest glands—totally haemorrhagic. The naked-eye appearances of these glands were particularly interesting to us since we have observed very similar changes in animals infected by us with *Trypanosoma gambiense*.

Experimental Inoculations

The following experimental inoculations were made in white rats, white mice, rabbits, and guinea-pigs, with the results indicated :—

Twenty white rats were inoculated with cerebro-spinal fluid or blood taken

either during life or *post-mortem* from patients admitted to hospital as cases of sleeping sickness and from cases of trypanosomiasis. Living trypanosomes in eleven instances were seen in the material inoculated. In seven of these the material inoculated was from cases of 'sleeping sickness,' in four from cases of simple trypanosomiasis. Of the former, three became infected; of the latter, two. None of the rats inoculated with material taken *post-mortem*, in which no living trypanosomes were seen, have ever become infected.

Four white mice were inoculated with cerebro-spinal fluid taken from two cases of sleeping sickness. Trypanosomes had been found in both cerebro-spinal fluid and blood of the first case, in the second trypanosomes were never seen. Two mice were inoculated with fluid containing many trypanosomes from the first case. One has become infected. Neither of the mice inoculated from the second case has ever shown parasites.

Two rabbits were inoculated with blood containing trypanosomes. The blood for one experiment came from a case of sleeping sickness, for the other from a case of simple trypanosomiasis. Neither animal has become infected. Four guinea-pigs were inoculated with blood containing trypanosomes, two from a case of sleeping sickness, and two from a case of trypanosomiasis. Both of the latter have become infected.

The very slight susceptibility of laboratory animals to infection with the trypanosomes found in man in the Congo, the great chronicity of the infection produced when inoculation has been successful, and the periodicity with which the parasite has appeared in the peripheral blood of the experimental animals, are points which greatly resemble the animal reactions of *Trypanosoma gambiense*.[6] The number of experiments done is not yet sufficiently large to permit the mention of incubation periods. During the eight or nine weeks which the infected animals have been under observation none of them have ever shown any gross sign of disease.

Conclusions

The examination of trypanosomes seen in stained specimens of blood from cases of trypanosomiasis, from cases of sleeping sickness (typical or doubtful), in the specimens of cerebro-spinal fluid of the latter cases, and in the blood of experimental animals, infected with either of three above-mentioned fluids, has led us to the following conclusions:—

1. The trypanosomes seen in the blood of man, whether symptoms of sleeping sickness were present or not, have always been the same. The number of cases in which trypanosomes from the spinal fluid have been examined is at present too small to permit of a definite description of morphologic characteristics. We have seen forms in the cerebro-spinal fluid similiar to those described by Bruce[3] and Castellani,[4,5] and also longer forms similar to those seen in the finger blood of the same cases.

2. None of the human trypanosomes seen, whatever their source, have presented any morphologic appearance incompatible with *Trypanosoma gambiense.*

3. The parasites seen in rats inoculated with the cerebro-spinal fluid of cases admitted to the hospital for sleeping sickness are the same as those seen in rats inoculated with the blood of cases of either sleeping sickness or simple trypanosomiasis.

4. The organisms seen in the blood of rats inoculated with trypanosomes from any of the three indicated sources have up to the present shown no differences from those observed in animals infected with *Trypanosoma gambiense.*

We have observed the extraordinarily long forms with prolonged flagella, and the stumpy forms with short flagella described as occuring in animals inoculated with the Gambian parasite.[6]

We have, therefore, no reason to suppose that the organisms seen by us in the Congo are other than *Trypanosoma gambiense.*

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1. Christy, T*he Epidemiology and Etiology of Sleeping Sickness in Equatorial East Africa*, with Clinical Observations, Royal Society Sleeping Sickness Commission, *Report III*, November, 1903.

2. Laveran and Mesnil, *Des Maladies à Trypanosomes, leur Réparition à la Surface du Globe*, Janus, August 15, 1903, pp. 395-402.

3. Bruce, Royal Society Sleeping Sickness Commission, *Report I*, August I, 1903.

4. Castellani, Royal Society Sleeping Sickness Commission, *Report I*, August, 1903.

5. Kruse, *Ueber das Trypanosoma Castellani den Erreger der Schlafkrankheit. Sitzungsberichten der Neiderrhein Gesellschatt f. Natur. u. Heilkunde zu Bonn*, May, 1903.

6. J. E. Dutton and J. L. Todd, *First Report of the Trypanosomiasis Expedition to Senegambia*, Liverpool School of Tropical Medicine. *Memoir IX.*

HUMAN TRYPANOSOMIASIS AND ITS RELATION TO CONGO SLEEPING SICKNESS

HUMAN TRYPANOSOMIASIS AND ITS RELATION TO CONGO SLEEPING SICKNESS

(Being the Second Progress Report of the Expedition of the Liverpool School of Tropical Medicine to the Congo, 1903) *

BY

J. EVERETT DUTTON, M.B., VICT.

WALTER MYERS FELLOW, UNIVERSITY OF LIVERPOOL

JOHN L. TODD, B.A., M.D., C.M., McGILL

AND

CUTHBERT CHRISTY, M.B., EDIN.

THE Expedition arrived at Leopoldville on November 21, 1903. Two of its members who had made a short tour through the Cataract Region arrived a week later. During their journey through this district, in which Congo Sleeping Sickness was said to be extremely prevalent, many cases, with very anomalous symptoms, but called sleeping sickness, were seen. As already stated in our *First Progress Report*[1] trypanosomes were found in the peripheral blood of some of these cases. It therefore became necessary to commence a clinical study of human trypanosomiasis in order to see what relation it bore to Congo Sleeping Sickness.

Leopoldville offered particular advantages for such a study, and the members of the expedition decided to remain there for a few months. A large bungalow was placed at their disposal by the Congo government, and through the kindness of Dr. GRENADE, the State Medical Officer, and Dr. BRODEN, Director of the Leopoldville Bacteriological Institute, the patients at the native hospital, a good proportion of whom were infected with trypanosomes, were given to the expedition to study. In addition to this the presence of about two thousand state employees—labourers, soldiers, etc.—afforded an easily accessible, healthy, native population.

Later, the hospital for natives was found unsuited for a complete examination of the cases, and a new structure was built and supplied with the necessary appurtenances through the kindness of the local government. The members of the expedition have therefore for four months been in a position to keep patients under continued and careful observation.

* Received for publication, May 23, 1904.

1. *British Medical Journal*, January 23, 1904

The following table indicates the results of examinations of cover-slip preparations of finger blood taken from four hundred and sixty-five natives at Leopoldville. The results of similar examinations, made in the region lying between Stanley Pool and the sea, are appended in order to present a concise idea of the prevalence of human trypanosomiasis in the Congo :—

Class of Native	Number examined	Number with Trypanosomes in Finger Blood	Number of Cases with Trypanosomes previously diagnosed as sleeping sickness
Healthy labourers, prisoners, women, and children, all resident for varying periods in or near Leopoldville, but many of whom are natives of distant parts of the Free State	255	6	0
Outpatients visiting the clinic of the State Doctor at Leopoldville and chosen for examination because of their miserable appearance	53	3	2
Patients admitted by State Doctor to native hospital	157	45	34
Total for Leopoldville	465	54	36
Total of previous examinations at Boma, Matadi, and in the Cataract Region .	707	49	21
Totals	1,172	103	57

In the British Colony of the Gambia only six cases of human trypanosomiasis were found among 1,043 natives.[1] These cases presented no definite symptoms of illness and nothing abnormal was detected, with the possible exception of an occasional rise in temperature and increase of pulse frequency.

In the Congo we have also seen examples of the mild Gambian type, but the majority of our cases have shown marked symptoms of illness. From a close study of these cases it becomes evident that there is no well-defined line of demarcation between these two forms of the disease, though for descriptive purposes we propose to consider them under three main headings, A, B, and C :—

Type A. Cases with no definite symptoms of illness.

„ B. Cases with few symptoms.

„ C. Fatal cases showing well-marked symptoms, the most notable being fever, lassitude, weakness, and wasting.

1. Dutton, J. E., Todd, J. L., *First Report of the Trypanosomiasis Expedition to Senegambia* (1902), Liverpool School of Tropical Medicine, Memoir XI.

It is significant to note that a large percentage—thirty-six out of forty-four of those classed under types B and C—were believed by their friends to be suffering from 'sleeping sickness.'

We have seen cases coming under type C in which no sleep symptoms were ever present, as well as cases in which some of the diagnostic signs of classical sleeping sickness were noted.

We therefore subdivide type C into :—

1. Fatal cases showing no sleep symptoms.
2. Fatal cases showing sleep symptoms.

Here again no sharp division is possible between these two groups, still, we think it not inadvisable to separate them, since, from our experience here we believe that altogether too much prominence, as a diagnostic feature, has been given to what appears to us to be only a minor and inconstant feature of this disease, namely sleep. The prominence given to this symptom has tended to disguise the true nature of Congo Sickness which is, primarily at least, a trypanosome infection.

The following charts and abstracted clinical reports will illustrate the characteristics of the Congo disease.

TYPE 'A'

Cases with no Definite Symptoms of Illness

Case 79. Fariala. Male. Age thirty.

History.—Admitted to hospital for chronic ulcer of thigh, January 7. Is from Lukila, Kasai district; has been for ten years in the neighbourhood of Leopoldville.

January 17. General condition. Is a well-nourished, sturdy man; intelligence good, answers questions quickly and well, shows no unsteadiness of gait, and complains of nothing save the ulcer. Skin is dry and dirty; there are several large scars and cicatrices on knees, ankles, and elbows, said to be due to injuries and burns. Face is pitted by smallpox. Slight oedema of right shin, probably due to old cicatrix.

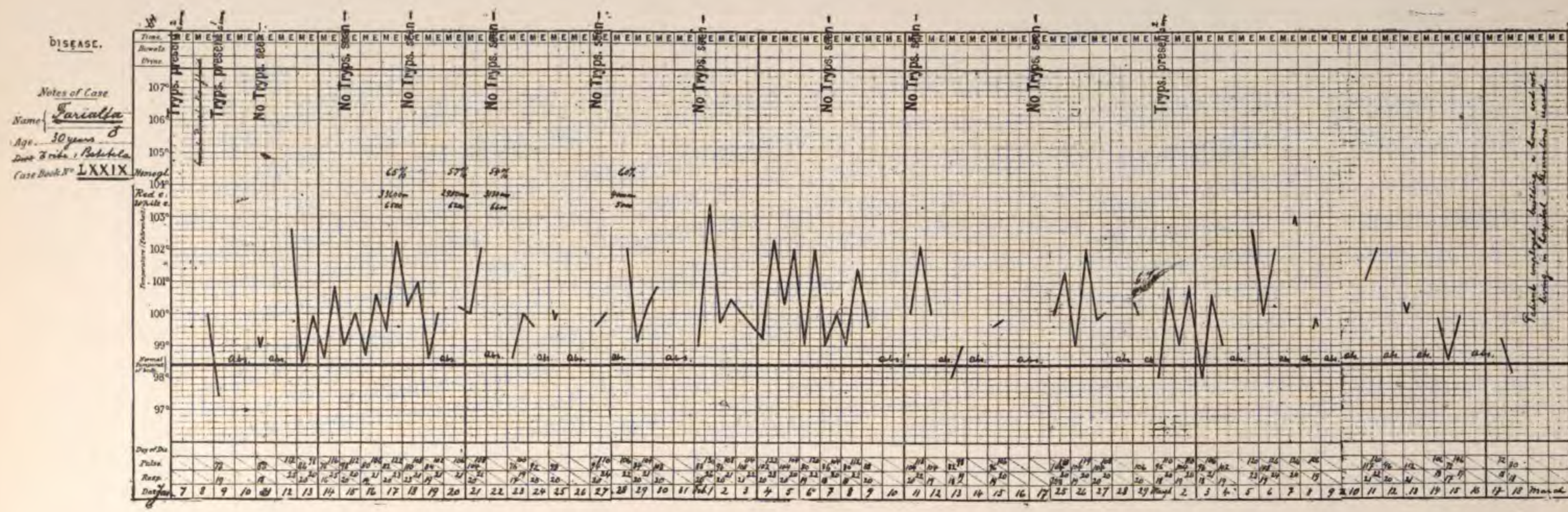
Lymphatic glands are all enlarged. Circulatory system: heart, position and dulness normal, loud mitral systolic bruit, aortic second sound accentuated. Respiratory system: normal. Alimentary system: tongue, teeth, and mouth normal; bowels regular.

Liver and spleen not enlarged. Nervous system: co-ordination perfect, superficial and deep reflexes normal, pupils react to accommodation and light.

January 25. Complains of nothing, and, save for ulcer, seems quite well. Blood shows filaria diurna, filaria perstans, and trypanosomes.

March 31. Patient at work, and apparently in good health.

CASE LXXIX


DISEASE.
Notes of Case
Name Farialla
Age 30 years
Case Book No. LXXIX
Tryps. present
Tryps. present
No Tryps. seen
No Tryps. seen
No Tryps. seen
No Tryps. seen
No Tryps. seen
No Tryps. seen
No Tryps. seen
No Tryps. seen
No Tryps. seen
Tryps. present
107°
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
65%
57%
54%
60%

Case 94. Kassongo. Male. Age twenty-three.

History.—A Batetela man. Has been for two years in Leopoldville as State labourer. Patient says that he never had fever.

February 23, 1904. General condition : Is a well-nourished, intelligent man, answers questions quickly and well. Has a stolid expression, but no dulness or vacancy whatever, actions not markedly slow. Oedema absent, no puffiness of face. Skin smooth and glossy. Lymphatic glands, all easily palpable, freely movable and hard ; parotids enlarged. Patient believes himself to be in perfect health. A careful physical examination reveals nothing abnormal in thorax or abdomen.

Nervous system. Co-ordination and sensation to touch and pain normal ; superficial and deep reflexes, normal ; pupils react to accommodation and light ; no tremors.

March 31. Physical examination repeated. With exception of a distinctly accentuated aortic second sound patient seems to be absolutely normal. He works willingly and well all day long.

TYPE 'B'

Cases, Intermediate Between Types 'A' and 'C,' showing very few signs of Illness, and not yet Definitely Believed by Their Friends to be Suffering From Sleeping Sickness.

Case 46. M'Pangila. Age seventeen. Male.

History.—Patient is a Lower Congo native. At age of thirteen he was admitted to the Baptist Mission Station at Wathen, Cataract Region. Here he was successively employed as garden boy, table boy, goat herd, and at the age of fifteen as cook. In December, 1902, he complained of fatigue while on the march, and was soon after dismissed because of untidy careless habits. His employers had at this time only a vague suspicion that these might be the prodromal symptoms of sleeping sickness. He had not since been seen until November 13, 1903, when, with another youth, he carried into the Mission a sick child from a village four-and-a-half hours distant. The only changes perceived in the lad were that much less care was taken of his personal appearance than formerly, and he was not so robust.

November 14, 1903. General condition. Patient is thin and has a certain, apathetic stolidity or dulness of expression. Oedema absent. Skin is soft and clean but dry. Lymphatic glands all considerably enlarged. Physical examination reveals nothing abnormal. Pulse, 96-110. Respiration, 20-24.

Temperature, with the exception of an evening rise to occasionally 99·5° F., is almost normal. Patient was sent to England, December 4, 1903. His condition since he has been under observation in Liverpool has not yet been ascertained.[1]

1. August, 1904, condition remains the same—parasites present in the blood.

Case 65. Mokoko. Male. Age twenty.

History.—Patient is a lower Congo native, and comes from a district in which sleeping sickness is present. He left his village a year ago, and has since been employed on a steamer plying on the Upper Congo. November 18, 1903, was admitted to hospital as a possible case of sleeping sickness. He says his illness commenced about the middle of September, with pain in his chest and knees, and a watery diarrhoea, no blood. These symptoms still continue.

December 12, 1903. General condition : Patient is very thin, muscles wasted. He is very weak and can only stand or walk with difficulty. He lies by the fire all day long without sleeping, and when spoken to insists that he has not 'sleeping sickness.' He complains of diarrhoea, and pain and tenderness in both hypochondria and epigastrium. There is no marked oedema. Skin is dry and dirty, slight 'craw-craw.' Lymphatic glands all slightly enlarged and hard. Respiratory system, slight bronchitis. Circulatory system, heart normal. Abdomen, distended and resonant. Liver, lower border not easily made out, apparently normal. Spleen, normal. Nervous system, co-ordination and sensation to touch and pain normal. Reflexes, superficial and deep, obtainable ; each muscle contracts to flicking ; pupils react to accommodation and light. Patient's condition seems to be wholly due to dysentery and bronchitis.

December 27. Functional murmur at pulmonary second sound.

January 5. Yesterday patient's blood showed over two hundred trypanosomes to the cover. His abdomen was distended and his feet oedematous, but there was little complaint. To-day the parasites are, perhaps, three times more numerous, the abdomen is more distended, and the feet oedematous. Patient complains of headache, pains in his joints, and of the abdominal distention. During these two days he has been more irritable, his breathing has been rapid and his skin moist. No murmurs.

January 6. No trypanosomes in blood, symptoms much alleviated. With the exception of the last two days patient has for some time been in much better condition than when he was admitted.

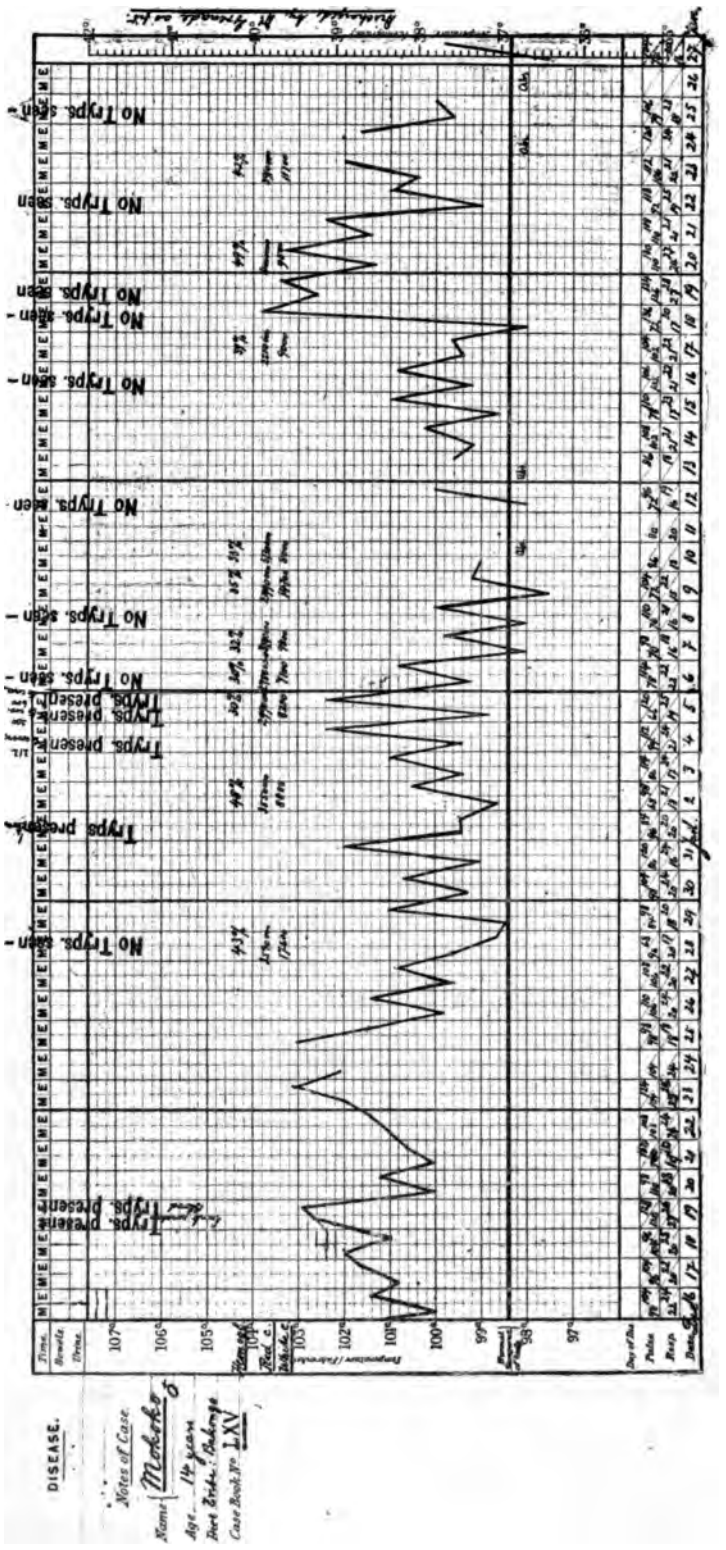
Faeces contain ova of *Anchylostoma duodenale*, *Ascaris*, and *Trichocephalus dispar.*

January 18. There is no oedema. Abdomen is distended and partially tympanitic. He complains of headache, but is otherwise comfortable. Is much stronger and strolls about.

January 28. Observations ceased, allowed to leave hospital.

February 13. Patient seen walking about with steady gait and with exception of abdominal distention and slight wasting, appeared to be well and expressed himself as such.

CASE LXV


DISEASE.
Notes of Case.
Name { Mokeke
Age 14 years
Port Evite: Bakenge
Case Book No. LXV
Trypanosomes / Fahrenheit
No Trypanosomes seen
Trypanosomes present

TYPE 'C'

Cases Showing Well-Marked Symptoms.

The two following cases illustrate sub-group 'I' of this type. Fatal cases showing no sleep symptoms.

Case 82. Kimfuta. Female. Age eight.

History.—Patient is a Lower Congo native, and comes from a village four hours' march from Leopoldville. When brought to Leopoldville three months back she was an orphan and destitute, and a fortnight ago was sent to hospital, more through lack of friends to look after her than because of any suspicion of sleeping sickness.

December 29. General condition. Patient is miserably thin, and has the appearance of being half starved. She is very weak, her feet are full of chiggers, and she can only walk with difficulty. She has a dazed expression, and is constantly trembling. Tremors are increased on the slightest exertion.

Conjunctivae are congested and there is a slight purulent secretion. Intelligence is good, answers questions quickly and well. Skin, dry, imbricated, and filthy. Lymphatic glands, all easily palpable and hard, save femorals, which are much enlarged, soft, and matted together. The child complains of backache, and insists that she has sleeping sickness, but her condition is thought to be due mainly to starvation and ill-treatment. Circulatory system, normal. Respiratory system: slight bronchitis. Liver, normal. Spleen, just palpable. Alimentary system: tongue heavily coated, teeth tartrous, gums healthy, appetite excellent, abdomen flat and hard. Nervous system, reflexes all increased.

January 8. In spite of attention, cleanliness, and better food, patient is going down hill.

January 16. Greatly emaciated, tremors increased, patient can hardly stand and cannot walk, speech is thick but coherent. There is no drooping of lids, but eyes are fishy and vacant; intelligence is now very dull; she whines and complains on being disturbed; eats little; is seldom asleep, although she is always lying down, and passes faeces under her.

January 19. Emaciation extreme, is too weak to rise, lies with wide-open mouth and eyes, whines, moans, or attempts to speak if touched.

January 20. Spleen punctured, only a few parasites seen, although blood contained 100 to cover. Died during night.

January 21.

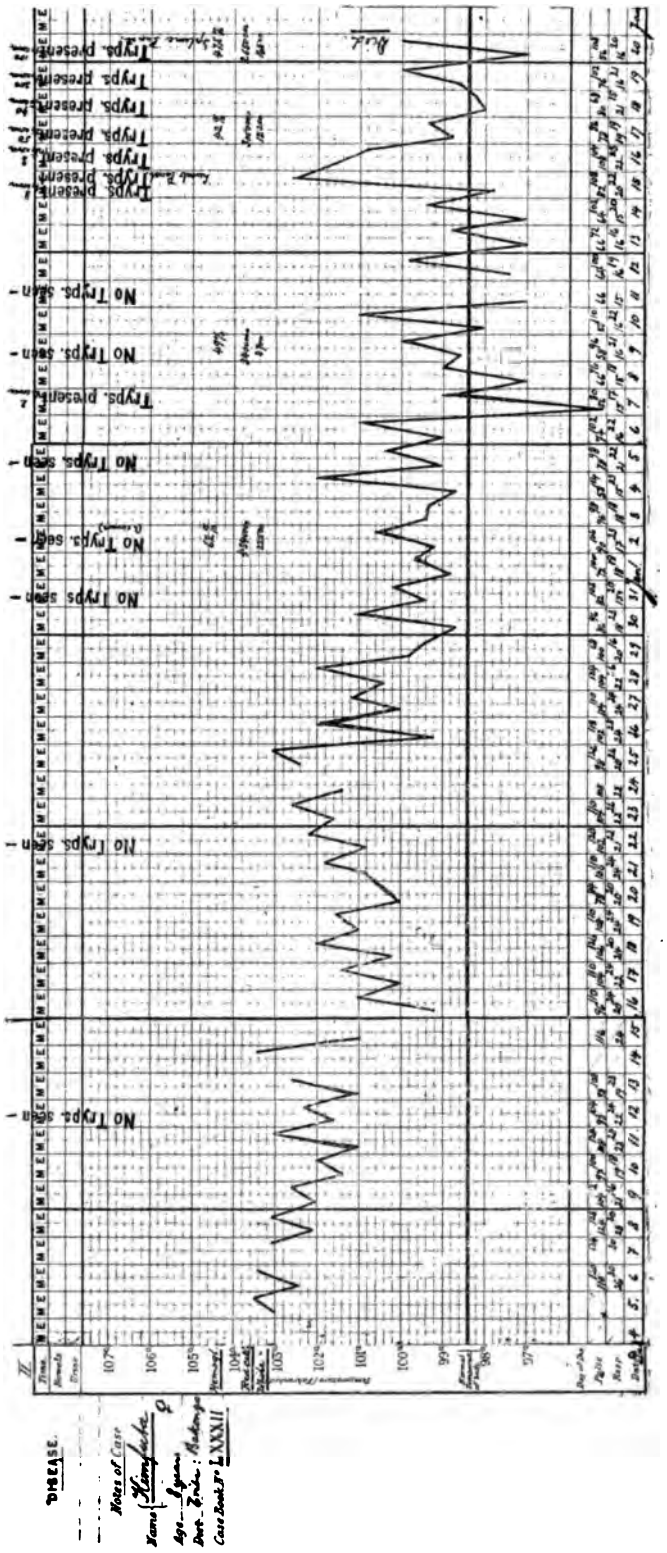
Necropsy commenced at 7 a.m., nine hours after death. Body much emaciated. No bed-sores, nor oedema. Ring-worm of scalp. Mouth foul, pyorrhoea alveolaris, teeth irregular, second dentition.

Thorax: Pleurae normal. Diaphragm: Right side, fourth space; left side, fifth rib.

Lungs: Left lung normal; right lung, old adhesions of upper and middle lobes.

Pericardium contained 5 c.cm. of turbid yellow fluid, in which no trypanosomes were found.

CASE LXXXII


DISEASE.
Notes of Case
Name: Mangbetu ♀
Age: Adult.
Dist. Tribe: Aakenga
Case Book: LXXXII
No Trypa. seen
Trypa. present

Heart dilated, both sides contained large, firm, white agonal clots. Valves normal. In left ventricle was small area of sub-endocardial petechial haemorrhage. Several pin-head calcareous nodules along cardiac veins.

Lungs : Weight, right, 227 grammes ; left, 151 grammes. Both show profuse sub-acute bronchitis ; bronchi full of mucous.

Abdomen : Omentum drawn up and wrapped around spleen, small clot of blood between it and outer surface of spleen due to puncture. Peritoneum contained no fluid. Old adhesions between transverse colon and gall bladder.

Liver : Weight, 682 grammes. Yellow, slight interlobular congestion. Gall-bladder full of dark-green semi-fluid bile.

Spleen : Weight, 228 grammes ; enlarged, anterior border very much notched ; substance very diffluent and dark red in colour. One small spleniculus.

Kidneys : Weight (together), 151 grammes ; normal. Bladder normal, filled with urine.

Genitals normal, save for left tube bound down in Douglas' pouch by firm fibrous adhesions Child has menstruated, remnants only of hymen.

Intestines normal, few anchylostomes in ileum.

Bone marrow (tibia), reddish orange in colour.

Brain : Dura not adherent to calvarium or pia. Superficial brain vessels much congested, vessels of basal ganglia very much so. There was fair amount of colourless, slightly turbid, sub-dural and sub-arachnoid fluid. Similar fluid occurred in ventricles and escaped from vertebral canal. No trypanosomes were seen in these fluids.

Lymphatic glands : Generally much enlarged, often oedematous, usually greatly congested and, in the abdominal and thoracic groups, haemorrhagic, sometimes excessively so. There were numerous patchy areas of dark sub-capsular pigmentation in the femoral and axillary groups.

Case 64. Dysiki. Female. Age twenty-six.

History.—Patient is from Lusambo, in the Kasai district, where sleeping sickness is said to be prevalent. She has been in Leopoldville for two-and-a-half years. Her illness is said to have commenced one-and-a-half months ago. Entered hospital because ' feet were sick and had difficulty in walking.'

December 8. General condition. Patient is very thin, expression somewhat dull and vacant, intelligence good, answers questions fairly quickly, muscles are wasted, and great weakness is apparent in her unsteady walk ; speech clear, but weak ; eyes very prominent ; lips puffy, dry, and cracked. Skin is dry, dirty, and scurfy. There is distinct pitting of shins and dorsa of feet but not of face or forehead. Lymphatic glands, all enlarged. Physical examination of abdomen and thorax showed no abnormality. Nervous system, co-ordination and muscular sense good, reflexes only just obtainable. Alimentary system : tongue steady, slightly furred, teeth and gums normal, appetite good, bowels regular.

December 15 to 19. Patient is very weak, sits most of the day outside the hut. Sleeps but very little, likes to sit in the sun.

December 21. Extremely weak, passes faeces in blanket without changing position. Prominence of eyes (ocular tension increased) and puffiness of eyelids and lips persists.

December 24. Patient in same state, helpless but conscious. Died during the morning.

Necropsy commenced one-and-a-quarter hours after death. *Rigor mortis* just commencing. Body thin, muscles wasted, eyes prominent, both pupils dilated (especially right). Oedema of shins, feet, and forehead. Marked cutaneous thickening of eyelids. Many chiggers in feet. Tongue bitten through, clenched between teeth (no history of a fit). Body very warm ; had been lying in sun, skin blistered. Panniculus scanty.

Thorax : Right pleura, few fibrous adhesions at base of lung. Left pleura, showed three small sub-pleural haemorrhages along vertebral column at level of fifth dorsal vertebra.

Pericardium contained 100 c.cm. clear yellow fluid.

Heart : Weight, 341 grammes ; fat, oedematous, valves normal, muscle pale, patchy thickening of endocardium of left ventricle, vessels normal.

Lungs : Slight bronchitis, oedematous. Weight : Right lung, 226 grammes ; left, 454 grammes.

Abdomen : Peritoneal cavity contained about 2 c.cm. clear fluid. Colon at level of umbilicus ; all abdominal blood-vessels turgid ; very extensive old fibrous pelvic adhesions ; several small broad ligament cysts full of clear fluid ; firm fibrous adhesions of liver and spleen to parietes.

Liver : Weight, 1,818 grammes : distinctly fatty, capsule over surface thickened. Gall bladder full of dark green fluid bile, ducts patent. Between liver and diaphragm was a layer, 5 c.cm. thick, of colourless gelatinous oedema.

Spleen : Enlarged and gorged with blood, substance soft and friable.

Kidneys, together, weighed 250 grammes, both showed cloudy change, capsules were adherent, and there was congestion of venae stellatae and around pyramids.

Pancreas and suprarenals normal.

Alimentary system : Mouth foul, gums soft, stomach normal, intestines normal, anchylostomes in jejunum.

Genitals : Vagina, slight mucous and cellular discharge, containing *Trichomonas vaginalis* and various bacteria ; no acute inflammation. Uterus nonparous, sub-acute metritis. Left ovary partially fibroid. Right ovary normal; no signs of recent inflammation in tubes.

Bone marrow (femur) : Very dark reddish orange.

Brain : Dura not adherent ; superficial vessels congested, but not so much as in many of the cases ; sub-arachnoid fluid not greatly increased and only slightly turbid ; ventricles contained a few c.cm. of yellowish, slightly turbid fluid, ependymal vessels congested ; no haemorrhages seen ; spinal cord vessels turgid.

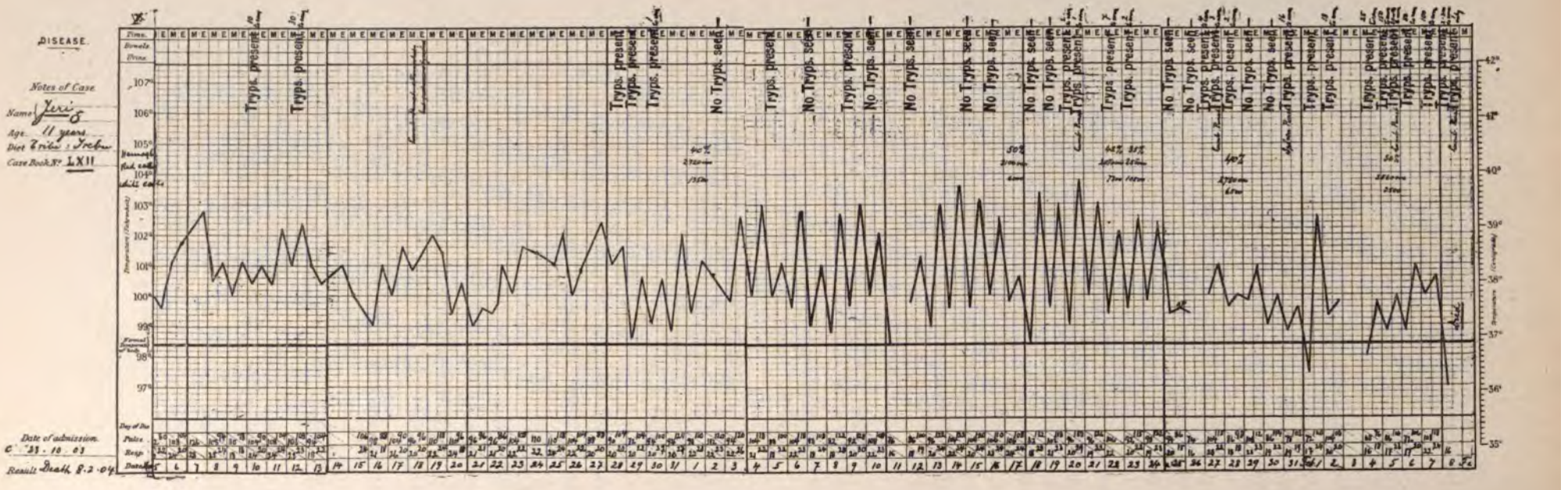
Lymphatic glands were nearly all enlarged, watery, and often congested. Some of pelvic and lumbar glands were chocolate colour and almost diffluent ; a small gland size of pea in much the same condition, but not diffluent, was found lying on head of pancreas. No actual haemorrhages into gland substance were seen. Fluids from pericardium, glands, receptaculum chyli, and oedematous tissues were examined, but no trypanosomes were found.

The following cases illustrate sub-group '2' of Type 'C.' Fatal cases showing sleep symptoms.

Case 62. Jeri. Male. Age eleven.

History.—Is a native of Irebu, a town near Lake Tumba, where sleeping sickness is said to be present. Left his village two years ago and has since lived in or near Leopoldville. He has been in hospital for one-and-a-quarter months. Was 'boy' to a white man who sent him to hospital as a suspected case of sleeping sickness. When

CASE LXII


DISEASE.
Notes of Case.
Name Yeri S
Age 11 years
Diet Tribe : Trefan
Case Book Nº LXII
Date of admission.
C 23.10.03
Result Death 8.2.04
Temp.
Bowels
Urine
Temperature (Fahrenheit)
Pulse
Resp.
Bandage
Day of Disease
Tryps. present
No Tryps. seen
Temperature (Centigrade)

first seen on November 23, patient was a thin, sharp-witted lad, and denied that he had sleeping sickness, but was said by his companions to sleep a great deal.

December 11, 1903. General condition. He is a weak emaciated boy; intelligence good; answers questions quickly; no thickness of speech; no unsteadiness of gait; is a mouth breather, and his 'adenoid look' gives him a certain vacancy of expression. Skin is dry and scurfy and covered with early 'craw-craw.' Muscles wasted; mucous membranes anaemic; lymphatic glands all enlarged. Circulatory system, normal. Respiratory system, slight bronchitis. Alimentary system, tongue steady, slightly coated, teeth and gums covered with tartar. Liver, 1 cm. below ribs. Spleen, 2·5 c.cm. below ribs, not tender nor painful. Nervous system, reflexes normal; pupils react to accommodation and light; co-ordination and muscular sense, normal.

December 28. Patient still answers questions quickly and well, has no pain nor headache, complains of a constant desire to sleep, but is seldom seen sleeping. Heart: reduplication of first apical sound. No oedema. Ocular tension is very distinctly increased, and the eyes are prominent. Knee jerks exaggerated, tendon reflexes not obtainable. He is able to go some distance from the hospital to collect firewood to cook his food. His inordinate appetite has become the joke of the hospital.

January 19. Patient now sleeps most of the day, or sits dozing over his fire with his head drawn back and his mouth wide open.

January 21. Is too weak to walk about, but lies in the hut complaining and talking of his weakness.

January 25. Sleeps much more than previously and is becoming, if possible, more emaciated. Is easily roused when touched.

February 2. Is vivacious and talkative again. When lying down or dozing over fire, head is drawn back and chin protruded to utmost as noted on January 19.

February 5. Is much worse again, apparently dying, lies curled up on his side, and is roused with difficulty.

February 8. Dying, almost pulseless, Cheyne-Stokes respiration, lies on back in an extreme state of opisthotonos, with legs extended and head drawn back to such an extent that two fists, one above the other, can be passed beneath him. Died at 7 p.m.

Necropsy commenced one-and-a-quarter hours after death. Body much emaciated, still warm. Slight oedema of forehead, shins, and dorsa of feet.

Thorax: Pleural cavities normal. Pericardium contains 100 c.cm. of clear yellow fluid in which were seen divisional forms of trypanosomes.

Heart: Weight, 226 grammes, normal.

Lungs normal.

Abdomen: No fluid, old firm fibrous adhesions of right lobe of liver to parietes.

Liver: Weight, 610 grammes, slightly congested, otherwise normal. Gall bladder full of dark thick bile. Ducts patent.

Kidneys: Right showed marked peri-pyramidal congestion.

Spleen: Weight, 266 grammes. Slightly enlarged, substance firm, not slatey.

Pancreas and suprarenals normal.

Intestines contained very many ascaris, anchylostomes, and a few *Trichocephalus dispar*.

Bladder showed two submucous haemorrhagic areas near meatus.

Genitals normal.

Lymphatic glands : Generally enlarged and watery, especially the abdominal groups, some of the latter were deeply congested ; one gland lying on the anterior surface of the pericardium had a distinctly haemorrhagic centre.

Brain : Slight congestion of superficial vessels and flattening of convolutions ; sub-arachnoid fluid in excess, 'ground-glass appearance' of arachnoid. *Cord* : vessels congested, great increase in cerebro-spinal fluid.

None of cranial bone cells were abnormal.

Living trypanosomes were seen in preparations made *post-mortem* from finger blood and pericardial fluid, but not in heart blood.

Case 84. Moidi. Female. Age twenty-four.

History.—Is a Kasai woman. Has been living for past two months near Leopoldville. Was sent to hospital, January 12, as a case of sleeping sickness.

January 21. Patient is a big well-made woman, no emaciation, her expression is dull, pained, and stupid, almost vacant. She is continually making grimaces. She cannot walk and lurches forward in a half drowsy condition during examination. Speech is thick and slow. Oedema of shins and feet, face and lips puffy. Indeed, the whole body is more or less puffy and presents an appearance of plumpness. Skin dry and dirty. There is a very extensive cicatrix, from a burn, implicating the right arm, side, and thigh. Lymphatic glands not enlarged. Circulatory and respiratory systems normal. Alimentary system, tongue coated and moist ; teeth and gums foul. Tip of tongue only, after much persuasion, protruded. Liver normal. Spleen slightly enlarged.

January 25. Patient usually sits dozing over a small fire. She is occasionally found lying at full length, naked, on the floor, or asleep in an uncomfortable attitude on the board bed. She can be easily aroused by a touch, and can, by shouting, be persuaded to speak.

February 5. Patient is almost lethargic, and is aroused only with some difficulty. She now eats nothing and is becoming emaciated. Sensation is dulled ; hardly flinched during lumbar puncture.

February 6. Died early this morning.

Necropsy six hours after death. The body of a massive but wasted woman. *Rigor mortis* present in masseters and extremities. Panniculus in fair amount.

Abdomen normal. Diaphragm : Right side, upper border fourth rib ; left side, fifth rib.

Thorax : Pleurae normal.

Pericardium contained a small amount of yellowish fluid.

Heart : Weight, 342 grammes ; contained firm white agonal clots.

Lungs : Weight, right, 454 grammes ; left, 335 grammes ; slight bronchitis and hypostatic congestion.

Liver : Weight, 1,591 grammes ; light in colour, central lobular congestion, soft and friable. Gall bladder full of dark, thick bile. Ducts patent.

CASE LXXXIV

DISEASE

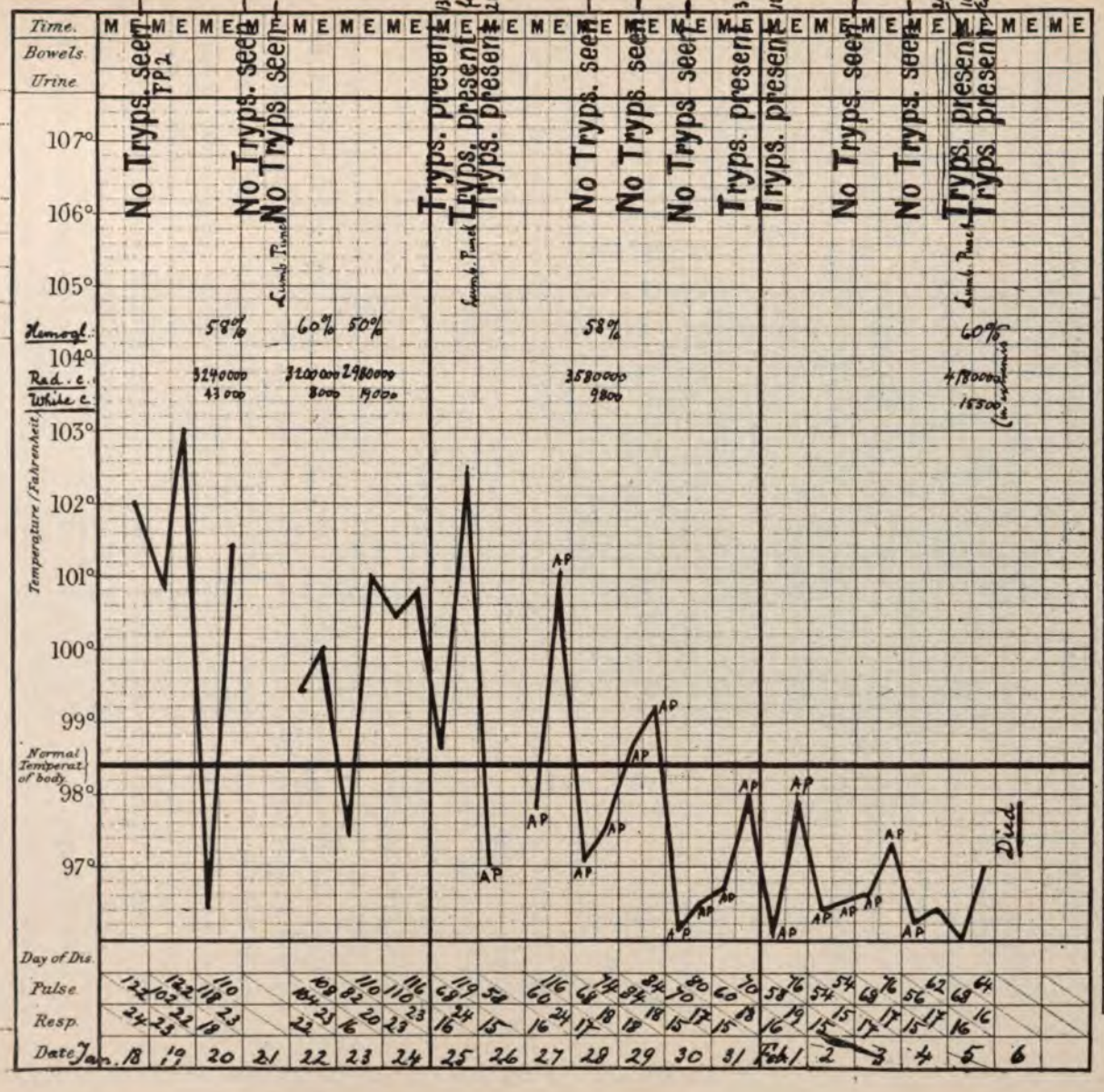
Notes of Case.

Name Moidi

Age ♀

Diet

Case Book N° LXXXIV



Date of admission
Jan. 12 1904

Result Death Feb. 6

Spleen : Weight, 345 grammes ; measures 22 × 10 × 4·5 cm. ; substance firm, slightly fibroid.

Kidneys : Weight together, 338 grammes, normal.

Genitals, with exception of chronic vaginitis, normal. Mouth foul with sores. Intestines contained a few anchylostomes and *Trichocephalus dispar*. Alimentary canal otherwise normal.

Brain : Vessels all much congested, sub-arachnoid fluid increased and turbid. Microscopically cerebro-spinal fluid showed pus cells but no trypanosomes.

Lymphatic glands all much enlarged. Retro-sternal and mesenteric glands slightly injected. Decomposition had commenced in retro-peritoneal glands. Femoral and inguinal glands congested and in one or two of the latter pus formation had commenced.

The two following cases, not easily classable under the foregoing headings, are, we think, of interest, as they illustrate the extremely rapid course the disease occasionally takes after symptoms have once developed.

Case 69. Kabali. Female. Age twenty-six.

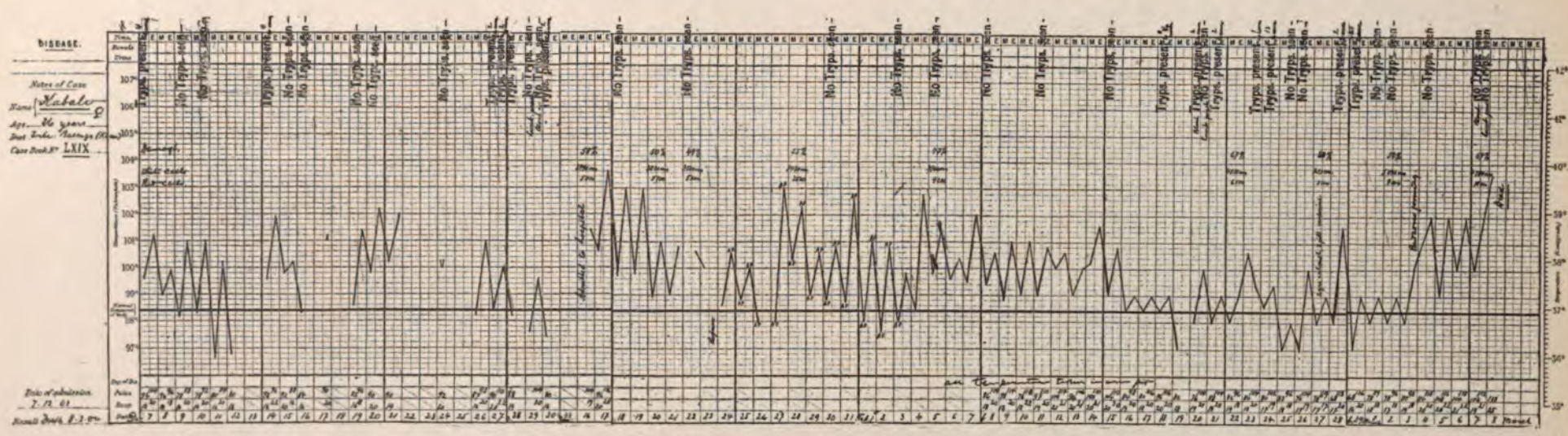
History.—Patient comes from the Kasai district, and left her native village, which is four days distant from Lusambo, ten years ago. Since then she has been in many places along the Upper Congo. Has lived in Leopoldville for the last two or three years.

When first seen on December 7, 1903, she stated that her illness had commenced four months previously, and began with rigor and fever ; the left side of her head swelled and was painful. There was no sore nor evacuation of pus. This condition lasted for six weeks. She says that she has since become thinner and weaker, particularly during the past two or three weeks. She has lost her appetite and has a tendency to diarrhoea.

December 25, 1903. General condition. Patient is a tall, well-developed woman, but thin, and her muscles are flabby. She is less active and vivacious than when first seen, and has a tired, listless gait. Her expression is rather dull, but she is intelligent and answers questions quickly and well. She speaks without hesitation or thickness. Complains of pain on pressure on shins. Skin is very abnormal. It is clean, though dry, and gives a rough feeling to the touch. This is most marked on shoulders, arms, and extensor surfaces of thighs and legs. In the parts most affected the skin has somewhat the appearance of an early exfoliative dermatitis, in which patches of glazed and thinned skin desquamate, slightly, at their edges along rectangular cracks and furrows. There is no itching nor discomfort, and the condition seems to be recent. At some spots are areas of crumpled tissue-paper-like wrinkles. No oedema. Lymphatic glands all enlarged. Tongue furred, moist, and steady. Circulatory system, pulmonary second sound is very much accentuated. Respiratory system normal. Liver normal. Spleen, considerably enlarged. Nervous system, co-ordination and muscular sense, normal ; reflexes, normal ; pupils react to accommodation and light, mental condition, alert.

January 18. Patient was admitted to hospital two days ago as a case of sleeping sickness. She is much thinner and weaker. Expression dull and sleepy. Skin

CASE LXIX


DISEASE.
Notes of Case
Name Kabale
Age No years
Case Book No LXIX
Date of admission 7.12.01
Enteric fever

condition less marked ; tongue coated and dry but steady ; complains of sleepiness and dozed during examination.

January 25. Lies in bed all day, is drowsy but does not sleep much, is irritable and querulous.

February 2. Seldom asleep, and then at once aroused, is bright and intelligent ; complains of pains 'all over' and of insomnia at night ; staggers with weakness on attempting to walk.

February 12. Is much weaker, can hardly walk ; speech is thick, weak, and monosyllabic ; lips flabby and glued together ; sleeps a good deal but easily aroused.

February 16. Lies in a drowsy condition all day ; complains that she cannot walk. Food is brought to her, but unless assisted she does not eat.

February 24. Sleeps lightly all day ; is almost too weak to sit up alone ; calls for assistance to go to stool.

March 7. Pulse rapid and weak ; patient has not slept during night ; complains of pains all over ; cries out when touched, owing to bed-sores forming on sacrum and scapulae ; pupils equal and widely dilated ; axillary and femoral glands enlarged, but not nearly so large as when admitted to hospital ; cannot take her food, either milk or soup. Knee jerks increased ; arms persistently flexed at elbow ; hands tremulous. No oedema.

March 8. Died 4 p.m.

Necropsy commenced three-quarters of an hour after death. Body much emaciated ; bed-sores over sacrum and both scapulae ; skin clean, dry, and shows slightly on extremities the previously noted 'imbricated appearance.' No oedema ; pupils widely dilated, left slightly more.

Thorax : Left pleura contained 30 c.cm., very turbid fluid with floating flakes of lymph, right pleura contained 10 c.cm. of similar fluid. Pericardium contained 40 c.cm. slightly turbid yellow fluid.

Heart : Weight, 223 grammes ; normal ; full of firm clot.

Lungs, each weighed 231 grammes. Marked hypostatic congestion, with sub-acute bronchitis of both lungs. At posterior border of left lung, level of seventh dorsal vertebra, there was a recent deposit of fibrous lymph on pleura, with intense subjacent congestion.

Abdomen : Peritoneum dry, mesenteric vessels congested ; spleen and liver adherent to parietes and adjacent organs by fairly extensive, old, fibrous adhesions.

Liver : Weight, 1,585 grammes, large, nutmeg, with commencing fatty change.

Gall bladder filled with thick grumous bile.

Spleen : Weight, 335 grammes, 19 × 11 × 4. 5 c.cm. Anterior border deeply notched, substance dark, firm, but friable.

Kidneys weighed together 225 grammes, normal.

Pancreas and suprarenals : Normal.

Alimentary canal : Mouth, stomach, large intestine, and jejunum normal. A Peyer's patch about 60 cm. above ileocaecal valve was greatly congested. On either side of patch for some 10 cm. were numerous disseminated congested areas, varying in diameter from 1 to 4 mm. Ascaris and anchylostomes present.

Genitals : Uterus small, nonparous small myoma. All genital organs firmly bound together by old adhesions ; vagina normal.

Brain: dura easily detached, sub-arachnoid space filled anteriorly with thick-formed lymph, posteriorly sub-arachnoid fluid very cloudy and semi-purulent; all vessels moderately congested. About 50 c.cm. turbid yellowish cerebro-spinal fluid escaped on opening tentorium. Cranial bone sinuses normal.

Bone marrow (femur): Very dark and diffluent, like clotted blood.

Lymphatic glands: All retro-peritoneal and pelvic glands very deeply congested; on section, soft, with a good deal of bloody fluid. One or two omental glands in a similar condition.

Glands from the other parts of the body enlarged but otherwise normal. Trypanosomes were not found by coverslip preparations in any of the body fluids or blood.

Case 86. Yaiyai. Female. Age twenty.

History.—Patient was sent to us on January 7 by native dispenser at a settlement near Leopoldville. She was said to have recently come from a district in which sleeping sickness was present. She was supposed to be in the 'initial stage' of that disease. Patient was well dressed, clean, and neat. She seemed in good health. She had, however, a peculiar vacancy of expression, and answered questions in an excitable and voluble manner, making grimaces. She stated that she had sleeping sickness, but, as yet, not badly. Patient was kept under observation for two days, but nothing abnormal was noted, save her expression and some display of irritability.

On *January* 24, she was carried into hospital in a listless, dazed, and sleepy condition, unable to walk, and apparently unable to speak.

February 4. General condition: Is a well-nourished woman; expression dull and vacant; manner apathetic and listless. Is able to walk with slow shuffling steps. Intelligence much dulled. Tongue coated and moist, slight tremors. Skin normal. Glands all enlarged save posterior cervical. Considerable oedema of shins and insteps; lips and eyelids puffy. Appetite good. Patient munches her food in a slow way, peculiar to many advanced cases. She frowns continuously. Circulatory, respiratory, and alimentary systems normal.

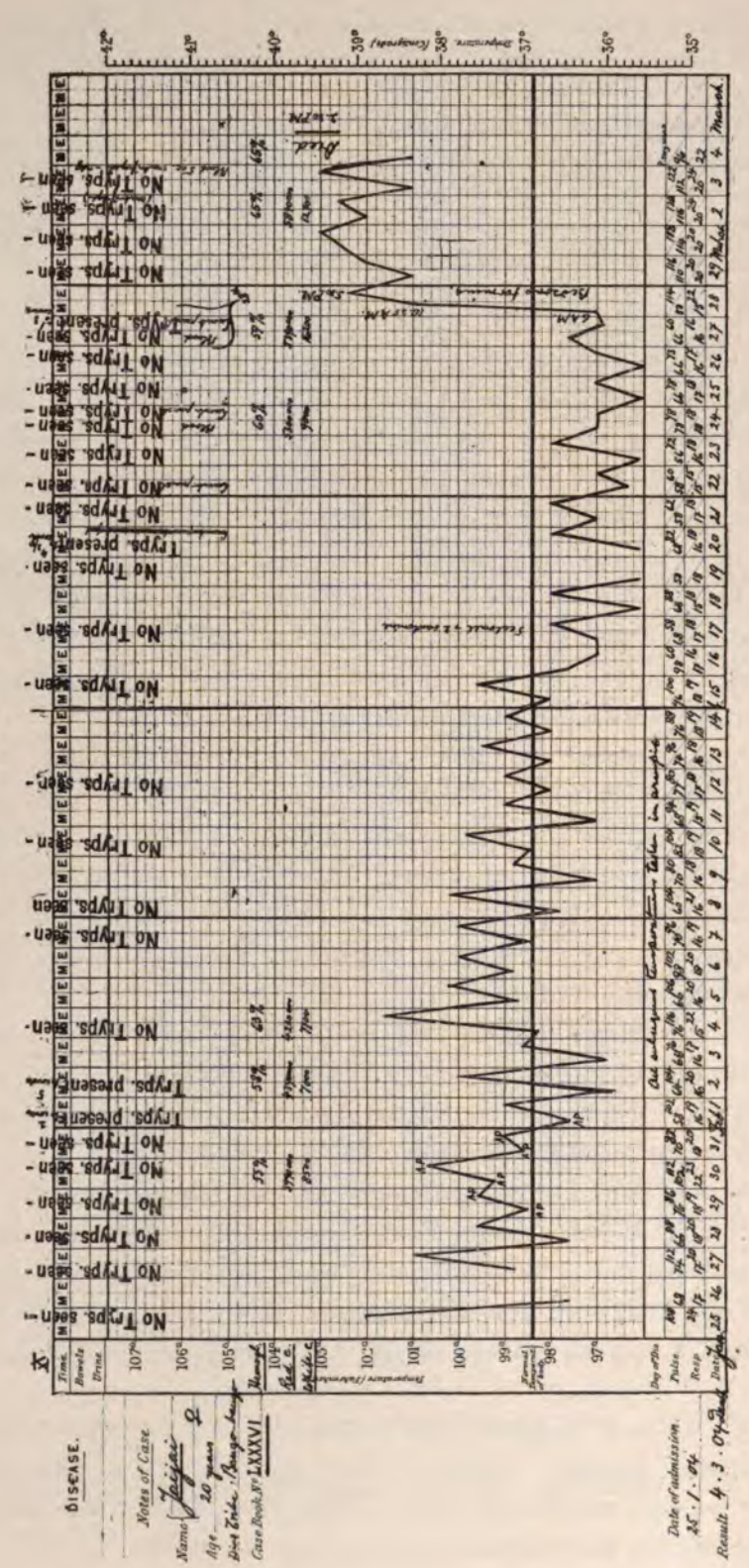
February 10. Sleeps a good deal but is much better than she was, is not so apathetic, and can talk and laugh while eating; appetite good.

February 16. Vacancy of face is again marked; patient is very weak and trembles all over; she speaks in weak voice only after much persuasion.

February 28. Since last note patient's condition has not altered. She has been unable to get off her bed; passes motions into bed; has only been able to take liquid food, and that only with assistance, is usually wide awake and conscious of all her surroundings, and, until now, has shown no very marked signs of wasting. To-day temperature, 102·4 F., profuse perspiration; crepitation and dullness at base of right lung.

March 3. Is now helpless, with sunken eyes and haggard face; body and limbs show marked wasting; oedema of shins and insteps and puffiness of face have disappeared; tremors, which were very marked, have ceased; she cannot take even soup; large bed-sores have formed on either side of sacrum.

CASE LXXXVI


DISEASE.
Notes of Case
Name
Age
Case Book, No LXXXVI
Date of admission.
Result
Temperature (Fahrenheit)
Pulse
Respiration
Urine
Bowels
Urine
No Trypanosoma seen
Trypanosomes present

March 4. Patient dying, but perfectly conscious, even acutely observant of all that goes on around her. Died at 2.30 p.m.

Necropsy commenced one-and-a-half hours after death.

Body warm, wasted though not distinctly thin. *Rigor mortis* just commencing, pupils evenly and widely dilated. Tissues dry, panniculus fairly abundant. Muscles normal.

Thorax : Pleurae normal.

Pericardium contained a few drops of fluid.

Heart : weight, 148 grammes, small, muscles pale and firm. Mitral valve showed distinct, reddish, gelatinoid thickening.

Lungs : Weight, right, 300 grammes ; left, 223 grammes. There was slight bronchitis of both lungs and marked hypostatic congestion, the latter condition marked in the right ; vessels contain firm, stratified clots.

Abdomen : Pelvic peritoneum congested and genitals firmly bound down by extensive fibrous adhesions.

Liver and spleen adherent slightly by old fibrous adhesions to adjoining organs and parietes.

Liver : Weight, 1, 582 grammes, slightly nutmeg. Gall bladder moderately filled with golden bile.

Spleen : Weight, 221 grammes, $17 \times 9 \times 5$ cm., upper lobe almost entirely divided by deep indentation from lower, substance dark, firm, friable.

Kidneys : Weight together, 218 grammes, normal.

Pancreas and suprarenals : Normal.

Alimentary Canal : Normal.

Bladder : Normal.

Genitals : Uterus small, non-parous, metritis with acute intense cervicitis ; fallopian tubes and ovaries bound down to uterus by old adhesions, acute vaginitis.

Bone marrow (femur) : Reddish brown.

Brain : Superficial vessels congested, particularly over posterior half of hemispheres (patient lying on back for several days before death). Relatively small amount, though increased, of sub-arachnoid fluid ; no ependymal haemorrhages ; about 30 c.cm. cerebro-spinal fluid escaped on cutting tentorium. Cranial bone sinuses normal.

Lymphatic glands : Retro-peritoneal and pelvic glands were enlarged ; the latter group being congested; the remaining groups of glands were normal.

In this attempt to illustrate the course of sleeping sickness, as found in the Congo, we have tried, as far as possible, to exclude from the illustrative cases those in which obvious secondary lesions were demonstrated *post-mortem*. It will be seen that deep sleep, continued sleep and lethargy, symptoms described as characteristic of sleeping sickness, are not features of the Congo disease as observed by us up to the present.

Trypanosoma Gambiense as a Probable Cause of Congo (Sleeping) Sickness

(*A*) As already indicated, in nearly every case in which sleeping sickness was diagnosed, or suspected, trypanosomes have been found in either blood, cerebro-spinal fluid, or both.

(*B*) We have shown that there is a very evident clinical connexion between those cases which have only very slight symptoms ('Trypanosoma fever') and the advanced cases of 'sleeping sickness' seen in hospital.

(*C*) In Gambia there is an undoubted trypanosome disease in horses which is characterized, in its first stage, by the absence of obvious symptoms ; and later, by fever, emaciation, and weakness. The course of this disease may be chronic and death delayed for a long period.

We have a horse, in good condition, under observation in Liverpool, which was first found to be naturally infected on October 30, 1902. A letter, dated January 18, 1904, from an officer in Gambia, says that his horse, found naturally infected in April, 1903, 'could not be better, and is in excellent condition.'

This Gambian horse disease seems to present a striking analogy to the human trypanosome disease seen in the Congo. The dull, listless expression, the weakness, the wasting and lack of energy, the irregular elevation of temperature, the frequent disappearance of the parasite from the peripheral blood (to ordinary examination), and the chronic course of the disease, are features characteristic of both infections. The similarity seems to us to be a point in favour of the casual relation of trypanosomes to Congo sleeping sickness.

(*D*) The sleeping sickness of the Congo is known to have a long latent period. Symptoms are said to have developed in some cases two to five years after leaving an infected locality.[1]

It is interesting to note that natives may be infected with trypanosomes for many months without showing signs of illness.

We have just heard that a Gambian native in whose blood trypanosomes were seen over a year ago is still in the very best of health.

Dr. ZERBINI, State Physician at Boma, has just sent us a report on cases observed by us in Boma during October last. His report is based on either his own observations or on the statements of heads of departments in those instances in which the cases had passed from his care. The report on five cases is indistinct or vague. Five had been in perfect health during the whole six months. Three have had surgical troubles, but are now well. One has had persistent diarrhoea, but has quite recovered and has been sent to the Upper Congo. Two cases have died of pneumonia, and the remaining four, only one of whom was in perfect health when we saw them, show slight oedema, lack of energy, or some other ailment. In none is there any suspicion of sleeping sickness.

(*E*) Europeans[2,3,4] infected with *Trypanosoma gambiense* show symptoms which are quite comparable with those observed in uncomplicated trypanosome infections of Congo natives.

(*F*) In Gambia, where cases of sleeping sickness are rarely seen (Dr. M. FORDE, Principal Medical Officer at Bathurst, stated that perhaps one case a year came under his care), only six out of one thousand odd natives were found to be infected,[5] by the examination of fresh cover-slip preparations. In an almost exactly equal number of a similar class of natives examined in the same way in the Congo, forty-six have been infected In Uganda, where the disease occurred in epidemic form, the percentage of infection among the general population was still higher.[6]

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1. Guérin, *Archives de Médecine*, VIe séries, vol. 14, Paris, 1869.
2. Manson, *Brit. Med. Jour.*, March 28, May 30, 1903.
3. Dutton, Todd, Christy, *Brit. Med. Jour.*, January 23, 1904.
4. Broden, Les injections à Trypanosomes au Congo chez l'homme et les animaux. (*Extrait du Bulletin de la Soc. d'études Coloniales*, Févr., 1904).
5. Dutton, Todd, *Ibid.*
6. Reports of the Sleeping Sickness Commission, Royal Society.

We have not yet met with an epidemic of sleeping sickness in the Congo, although at least one has been recorded.[1] From the answers to inquiries, which we have received from state doctors and officials in different parts of the country, we do not think that the disease exists in any part of the Congo in a much more severe form than at Leopoldville, and certainly there is no epidemic comparable to that in Uganda.

It will be noted that the Congo type of case, as we have described it, bears a close resemblance to some of the cases described by BRUCE and NABARRO on the Victoria Nyanza, at a time many months after the epidemic wave had passed eastward, and at a place Entebbe, situated at the extreme tail of the epidemic area. Moreover, one of us is able to recognize a similarity between many of the cases seen here, particularly those in which emaciation, lengthened period of illness, and absence of sleep, are the main features, and many of the cases seen by him on Buvuma Island, also situated in Victoria Nyanza, and towards the tail of the epidemic area.[2]

THE COURSE OF THE DISEASE

Duration. It has been impossible to decide for how long a period a native may be infected with trypanosomes and still show no definite signs of disease. One man already mentioned, is known to have been infected for over a year and is still perfectly well. It would appear from one or two early cases which have been observed that the transition from this 'latent' stage to one in which symptoms are noticed may be very gradual. We have only observed eight fatal cases of Congo sickness in which the necropsies showed no obvious secondary infection. The duration of the disease, dating from the recognition, either by the friends of the patient or by ourselves, of obvious signs of ill-health, has been from two to four months.

Recovery. In our experience no native who has shown definite and constant signs of ill-health has recovered.

Since we have been in Leopoldville we have frequently seen a case of trypanosomiasis in a European, reported by Dr. BRODEN[3] and Sir PATRICK MANSON.[4]

Mrs. M., a missionary stationed at Leopoldville, states that her illness commenced on October 1, 1900, with a fever which lasted for three months and was not amenable to quinine. Since that time she had not been free from constantly recurring fever, except for two periods, in 1901 and 1902, each of three months duration, up to March 19, 1903. Since the last date she has been absolutely free from fever or other signs of trypanosoma infection up to the present (April, 1904). She is apparently in perfect health and has increased in weight. The trypanosomes were found by Dr. BRODEN on February 7, 1903, during an attack of fever which lasted for a few days. During the last year Dr. BRODEN has seen no parasites in the blood of this case.

Death. We do not think that we have sufficient evidence to state that death is produced by the trypanosomes alone.

1. Bergh St. Marie in the Portuguese Commission's Report.
2. Christy, Reports of the Sleeping Sickness Commission, Royal Society, No. III.
3. Broden, *Ibid.*
4. Manson, *Ibid.*

Secondary Infections and Complications

Secondary bacterial infections seem to determine the fatal issue of many cases of Congo sleeping sickness. Thirteen out of twenty-two necropsies performed at Leopoldville showed complications or obvious secondary infections.

They were as follows :—

Purulent meningitis	4
Purulent pleurisy and pneumonia	1
Pneumonia and localized tubercle of lung	1
Localized gangrene of lung	1
Enlarged caseating and breaking down glands in thorax and abdomen. No tuberculous lesions in organs (tubercle bacillus in glands seen in one case)	2
Dysenteric ulceration of large bowel (perforation in one case) ...	2
Universally adherent pericardium (recent)	1
Infiltration of pus in femoral, inguinal, and internal iliac glands (gonorrhoea)	1

According to the *Post-mortem* Reports of Colonel D. Bruce and Dr. Nebarro very similar lesions were observed in Uganda, where ten out of twenty autopsies on sleeping sickness patients showed obvious secondary infections and in three instances purulent meningitis.

It will be noted that purulent meningitis has been the most frequent complication both in Uganda and in the Congo. The brain in these cases (see case 69) presents a very abnormal appearance. The convolutions, especially on the upper surface, are covered and glued together by a thick layer of tenacious lymph, and the vessels of the pia arachnoid are intensely congested. It is evident that such morbid changes are explicable on bacteriological grounds, and we have found, microscopically, in all such cases an almost pure culture of a diplococcus occurring in small chains. These observations make us doubt whether the acute congestion of cerebral vessels accompanied by an increase of pia arachnoid fluid containing pus cells, seen by us here, and described by others as typical of sleeping sickness, can be attributed to the trypanosome alone.

The Portuguese Sleeping Sickness Commission,[1] Dr. Broden in Leopoldville,[2] and Dr. Castellani in Uganda,[3] have all described bacterial infections in a very large percentage of sleeping sickness cases examined before as well as after death.

It must be noted that such purulent changes have not been described in animals dying from other trypanosome infections.

1. Bettencourt, Koppe, de Rezende and Mendes, *La Maladie du Somneil*, 1903.
2. Broden, *Ibid.*
3. Castellani, Report of the Sleeping Sickness Commission, Royal Society.

OBSERVATIONS ON THE PARASITE

Periodicity and Frequency of Occurrence in the Peripheral Blood

Frequent examinations of the finger blood of trypanosome cases have shown that the parasites may, to the ordinary methods of examination, be absent from the peripheral blood for varying periods. This periodicity is illustrated by the two following cases :—

Case 101. Oporanga. Regular observations were made about every third day, commencing on January 3, 1904, when no parasites were seen. The periods of presence or absence of the parasite from that date until death were as follows :—

22	days	absent	...	11	observations.
3	„	present	...	3	„
7	„	absent	...	4	„
3	„	present	...	3	„
33	„	absent	...	14	„
4	„	present	...	4	„
6	„	absent	...	3	„ Died.

This case shows comparatively long periods of absence.

Unless especially stated the routine method of examination employed for the detection of the trypanosomes was the examination of a freshly made three-quarter inch square, cover-slip preparation of finger blood ringed with vaseline.

Case 90. Bongwendie. Routine examinations commenced on January 19, when trypanosomes were found to be present. They had not been seen for four days previously.

3	days	present	...	3	observations.
9	„	absent	...	4	„
6	„	present	...	4	„
6	„	absent	...	4	„
1	„	present	...	1	„
2	„	absent	...	1	„
1	„	present	...	1	„
5	„	absent	..	4	„
2	„	present	...	1	„
3	„	absent	...	1	„
6	„	present	...	5	„
10	„	absent	...	7	„ Died.

This case shows shorter periods of absence.

In some cases parasites have been almost constantly present in the blood (Case 85); in others, as is shown by the two following cases, they have been rarely found.

Case 77. Ejoli. Under observation for twenty-one days before death. Parasites seen only twice on two consecutive days; sixteen observations.

Case 103. Belamo. Still under observation. Parasites not seen for thirty-eight days; seventeen observations. They have been seen in the blood of this case only by centrifugalizing.

The frequency with which the parasite is obviously present in the peripheral blood bears no relation to the severity of the symptoms.

Number of Parasites Appearing in the Blood

The number of parasites seen in ordinary fresh cover-slip preparations is generally small, but in some patients, very large numbers have been recorded. In two cases a large increase has occurred during the few days immediately preceding death.

Case 87. Patesa. Admitted to hospital for acute dysentery; under observation for four days preceding death. Parasites increased from two to a cover on the first day to twenty to a field on the day of death. There was no accompanying rise of temperature.

Necropsy showed very extensive dysenteric ulceration of the large intestine.

Case 88. Boyo. Parasites increased during the four days preceding death from two to one hundred to a cover. They had not been previously seen in such large numbers. At the necropsy on this case very general enlargement and caseation of abdominal and thoracic glands was noted. There were no tubercular lesions in the lungs or other organs.

A similar increase of parasites occurred just before death in Cases 62 and 82 (see charts); but in the two following cases they were absent for some days before death :—

Case 89. Kapinga. Parasites not seen for ten days preceding death; six observations.

Case 77. Ejoli. Parasites not seen for nineteen days before death; fourteen observations.

Sudden Disappearance of the Parasite from the Finger Blood

The following are examples of cases in which the parasites have gradually increased from small to fairly large numbers (twenty to cover-slip or more) and then suddenly disappeared on the day after their acme was reached.

Case 65. Mokoko. See chart.

Case 99. Mozao. At the end of the first examination on March 5 seven trypanosomes were seen to a field. On March 6, 7, and 8 none were seen.

Case 102. Kondolo. On February 24, seven parasites were seen to a cover; on February 25, seventy to cover; on February 26 and eight following days, none were seen, although observations were made every other day.

A second similar acme and sudden disappearance was recorded in this case. On March 10, six parasites were seen to cover; on March 11, two hundred and fifty; but on March 12, none were found.

Case 90. Bugwendi. On February 26, 27, 29, and March 1, parasites were seen in the following numbers respectively, eight, five, five, and one to a cover. On March 2, twenty-four were seen; but from March 3 to date of death (March 21) they were absent.

Case 101. Oporanga. On March 1, 2, 3, parasites were seen in numbers six, forty-eight, thirty-one, respectively, to the cover-slip. On March 4, one hundred and twelve were noted; but on March 6, 7, and 8, not one was to be found. (No examination on March 5).

Case 66. Tenda. On February 23, 24, and 25, the number of parasites recorded per cover was four, five, and seven, respectively,. On the 26th, seventy to a cover; but on the 27th, and for fifteen subsequent days, none were found.

Case 85. Banja (see below). Illustrates a somewhat opposite condition. The parasites increased in this case to an acme which lasted for two or three days, and then a marked diminution in number was observed. This patient, who is still under observation, illustrates the constancy with which parasites are seen in the peripheral blood of some cases. It will be noted that his general health has latterly improved.

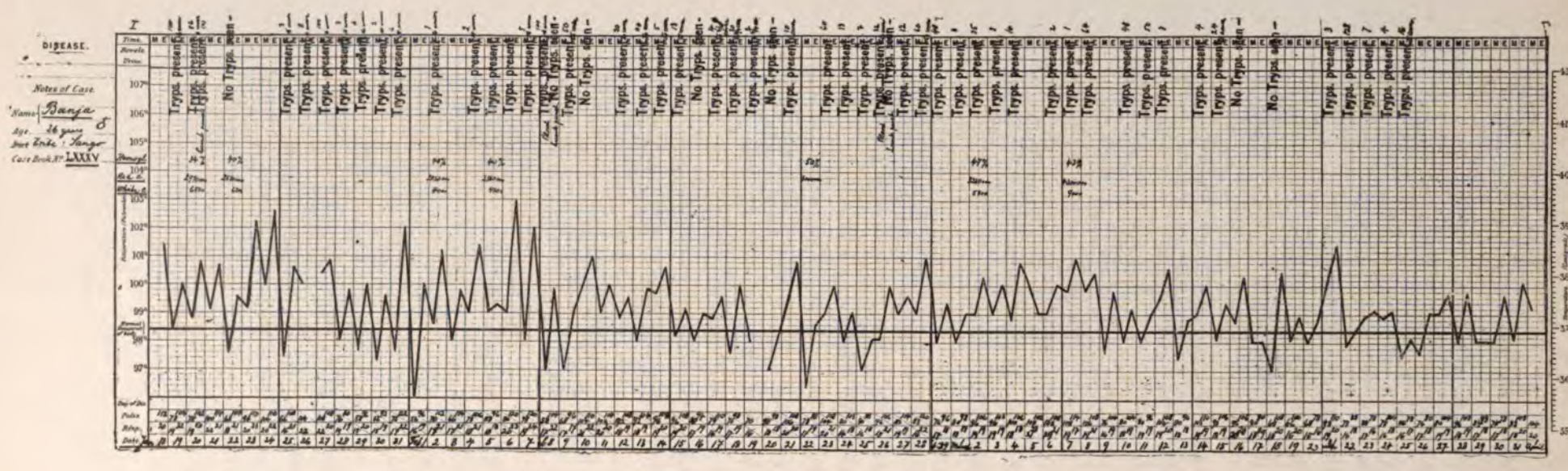
Case 85. Banja. Male. Age twenty-six.

History.—Patient comes from the Sango district of the Bangala country, where Congo Sickness, so far as we know, is not present. He has been employed for some years on steamers plying on the upper river. A month before being sent to hospital was imprisoned on a charge of cannibalism. No history of illness before going to prison.

January 20, 1904. General condition: Somewhat emaciated; expression pained and anxious; intelligence fair, answers questions slowly but correctly; breathing laboured—is apparently very ill. His skin is dry and filthy, no 'craw-craw.' Slight oedema of forehead, considerable of shins and feet; lymphatic glands all enlarged, especially femoral and inguinal; tongue coated, moist, and steady.

Circulatory system, heart sounds booming at apex; pulmonary second sound accentuated and dicrotic. Respiratory system, dullness at base of right lung, loss of breath sounds, pleuritic rub and moist rales. Liver enlarged and painful. Spleen not enlarged. Nervous system, patellar and cremasteric reflexes not obtainable, epigastric increased; pupils equal and react to light.

CASE LXXXV


DISEASE.
Notes of Case
Name Banja
Age 16 years
Last Entd: Sango
Case Book, P. LXXXV
Trypa. present
No Trypa. seen
Temperature (Fahrenheit)
Temperature (Centigrade)
Pulse
Resp.
Date

February 2. Is in better condition, and is brighter than when admitted; abdomen distended; oedema of loins marked.

February 16. Shows no signs of sleep, walks now without any unsteadiness. Appetite large.

February 24. Seems quite well; slight oedema of shins, but none elsewhere; no puffiness of face; can walk about with ease.

March 18. Gait normal, expression contented, no dulness, is quite intelligent, answers questions quickly and well; glands all enlarged, but femorals and inguinals apparently smaller than when admitted to hospital; slight oedema of shins; liver still enlarged, but not tender; heart and lungs, normal; knee jerks, normal; epigastric and cremasteric reflexes obtainable; appetite good; no drowsiness; patient works willingly around the hospital.

April 1. Has certainly put on flesh lately and increased in general robustness.

Symptoms Associated with the Presence of the Parasites in the Blood

The only patient in whom a large increase of parasites was associated with a rise in temperature and the presence of symptoms which might be attributed to the parasite was Mokoko (Case 65), whose case is given above.

From our observations we cannot make out any definite relation between the temperature and pulse and the appearance of the parasites in the peripheral circulation. A rise of temperature is not necessarily associated with an increase of parasites in the blood.

It appears, therefore, that the number or constant presence of the parasites in the peripheral blood bears no relation to the severity of the disease.

Occurrence of Parasites in Serous Fluids

On two occasions 10 c.cm. of fluid was drawn from a flabby hydrocele (in Case 104) and centrifugalized. Parasites were seen each time, although they were absent from the blood to ordinary examination, and probably had been absent for some days previous to the second tapping. The cerebro-spinal fluid of this case was also examined on the same date as the first tapping, but no parasites were seen, although the deposit of the centrifugalized hydrocele fluid showed fourteen to a cover. In another case (number 92), in which the penis and scrotum were very oedematous and there was a small right hydrocele, no parasites were seen in either oedema or hydrocele fluids, although the blood showed thirty to a cover. We have not found parasites in the urine (centrifugalized) in the few cases examined in which the blood showed many parasites.

The Occurrence of the Parasites in Tissues and Fluids after Death

Our examination of film preparations of tissues and fluids taken at autopsies are not completed, but it is interesting to note here the frequency with which the parasites have been seen in the pericardial, pleural, and peritoneal fluids when examined fresh. In three cases (62, see chart, 64, 76) parasites were seen, without centrifugalizing, in the pericardial or peritoneal fluid; one-and-a-half, two, two-and-a-half hours, respectively, after death. In two of these cases numerous longitudinal divisional forms were seen in the pericardial fluid. In three other cases actively motile trypanosomes were seen in the pericardial fluid after centrifugalizing, fourteen, fifteen-and-a-half, twenty-and-a-half hours after death. In all these cases parasites were seen either the day before or a few days previous to death. In only one case has a trypanosome been seen in fluid from a lymphatic gland (omental); but in this case parasites were easily detected in the blood, *post-mortem*, and were very numerous in the pericardial and peritoneal fluids.

Occurrence of Parasites in the Cerebro-Spinal Fluid

During the stay of the expedition at Leopoldville, lumbar puncture was performed on forty-nine natives coming from many parts of the Upper and Lower Congo. Of these thirty-eight were proved to be suffering from trypanosomiasis, the parasites being found in the blood. In the remaining eleven cases no trypanosomes were seen in either the blood or cerebro-spinal fluid, and in some of them a diagnosis of some other disease, *e.g.*, tubercle, dysentery, etc., was established either during life or *post-mortem*.

In twenty-five of the thirty-eight trypanosomiasis cases the parasites were found in the cerebro-spinal fluid, but in thirteen no parasites were seen, although in one case (No. 101) the fluid was examined on five occasions.

If, however, those punctures in which the cerebro-spinal fluid was mixed with the blood in greater proportion than from three to four red cells to a field (Zeiss 1/6 objective, No. 4 eye-piece, diaphragm removed), and in which the parasites were found by coverslip examination to be present in the peripheral blood on the same day as the puncture, be excluded, then we find that the above result is very different, namely, thirty-two cases, in sixteen of which the parasites were found in the cerebro-spinal fluid, and in sixteen they were not. The amount of cerebro-spinal fluid drawn off and centrifugalized at each operation varied from 10 to 30 or even 40 c.cm. This, if necessary, was not only centrifugalized a second or third time, but from one to six coverslip preparations of the resulting deposit were examined before a negative result was recorded.

If the cerebro-spinal fluid is mixed with blood it has a slight yellowish tinge, and is opalescent or clouded according to the amount of blood it contains. If

normal cellular elements are much increased the fluid has also an opalescent or cloudy appearance, but there is no yellowish tinge. It is doubtful whether the presence of the parasite in the cerebro-spinal fluid has any influence upon the increase of its cellular elements. It would seem, from a study of our cases, that the fluid as a rule, whether the parasites be present or not, is perfectly clear and limpid as in health. Only in a few instances, in both positive and negative cases, have the cellular elements shown an apparent increase, consisting mainly of small mononuclear cells, together with some mononuclear large cells. In Case 93, the only one in which a large number of parasites—fifty to a cover—were found in the cerebro-spinal fluid, a great increase of small mononuclear cells was noted at the same time. A month later the cerebro-spinal fluid was again examined, but only one parasite could be found, and the cellular elements were scanty. This case, up to the present date, nearly two months after the discovery of large numbers of trypanosomes in the cerebro-spinal fluid, has shown no tendency to sleep during the daytime, no wasting, nor any of the more noticeable symptoms associated with the later stages of many of our cases of trypanosomiasis.

As a rule, we have found that the parasites occur in extremely scanty numbers in the cerebro-spinal fluid, seldom more than one to three or four, very occasionally from ten to twenty to the cover-glass preparation of the sediment left after centrifugalizing.

As already stated, neither at Leopoldville, nor anywhere on the Lower Congo, up to the present time, have we met with a case of Congo sickness in which sleep has had a prominent place among the clinical symptoms, and in those few cases in which it has been noted a few days before death, or at irregular intervals during the course of the disease, it has not been describable as deep or continuous sleep, but merely a drowsy or somnolent condition from which the patient was at once aroused by being touched or, perhaps, by being spoken to. On comparing the cases in which parasites were found in cerebro-spinal fluid with those in which they were not found, we see that most of the few cases in which drowsiness was a feature, together with cases in which head symptoms, *e.g.*, mild mania, epileptic attacks, flexure contractions, and convulsive seizures, were conspicuous are upon the positive side. On the negative side similar cases are also found, but it is noticeable that there are many of those cases which showed hardly any symptoms, and which, if no fatal complications intervened, lived on month after month in an advanced state of emaciation, retaining their faculties, speech, appetite, etc., almost to the moment of their death.

It is, therefore, not improbable that the presence of the parasites in the cerebro-spinal fluid at a late stage in the disease may tend to increase the gravity of the case by predisposing to one or other of many complications, or, in other ways, hasten a fatal termination ; but that this is not invariably so is proved by at least two of our cases, one of which (Case 93) is now one of the most sturdy patients under observation.

Animal Experiments

We have infected rats, mice, guinea-pigs, rabbits, and monkeys with human trypanosomes taken from cases of Congo Sleeping Sickness, both in its early and its latest phases. As was stated in our first Progress Report the course of the infection in these animals has given us no reason to suppose that we are dealing with more than one species of trypanosomes, or that the parasite is other than *Trypanosoma gambiense.*

All our inoculations have been made with small or medium doses (1 to 5 c.cm.) of infective material, either cerebro-spinal fluid or blood—diluted or no—in which, in almost every case, living trypanosomes, sometimes in enormous numbers, were demonstrated. About 50 per cent. of such inoculations have failed to infect. These failures seem to bear no relation either to the source of the material inoculated, to the site of inoculation—subcutaneous or intraperitoneal—or to the approximate number of parasites injected.

Once more, no animals inoculated with material in which trypanosomes were seen, taken *post-mortem* from cases of trypanosomiasis, have ever become infected. Parasites have been seen in the peripheral blood of infected animals only at more or less irregular intervals. Guinea-pigs have, perhaps, shown themselves the most satisfactory of ordinary laboratory animals, since, as a rule, when once infected, parasites are constantly present in large numbers.

Death, in the small number of our infected animals which have died, can in no instance be certainly said to have been due to the trypanosome alone. These are all points in which the Congo trypanosome resembles *Trypanosoma gambiense.* In addition, the 'incubation period,' that is, the period intervening between the inoculation of small or medium doses of infective material and the detection of the parasite in the peripheral blood of the experimental animal, is much the same for both parasites.

	Congo trypanosome	Trypanosoma gambiense
Rats	5—20	7—20
Mice	5—16	6—7
Rabbits	17—27	21
Guinea-pigs ...	11—25	12—21

Monkeys of two different varieties, both belonging to the sub-family *Cercopithecus*, have been infected. Only one has shown slight symptoms of ill-health. On two

Fariala (Case LXXIX). Illustrating an early stage with general absence of symptoms except rise of temperature.

Two cases from Cataract Region of Congo, showing practically no symptoms. Carried heavy loads for many days without complaint.

Trypanosomiasis cases in the hospital for blacks at Leopoldville, Upper Congo, waiting for their daily ration of 'Kwanga' (Casava bread) to be served out to them.

To follow page 45

occasions a slight rise of temperature and loss of vivacity has accompanied one of the irregularly appearing intervals during which parasites have been seen in the peripheral blood. This monkey has been infected for nearly three months.

We have again failed, even with large doses of blood containing huge numbers of trypanosomes, to infect two dog-face monkeys (*Cynocephalus*).

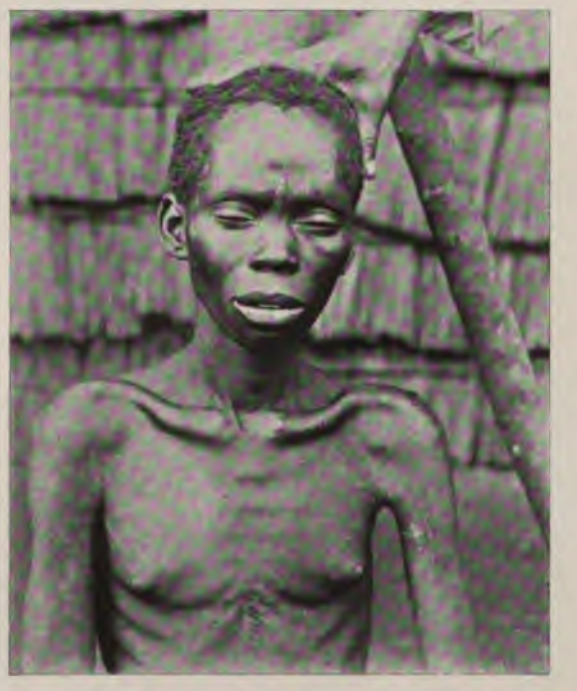
Transmission Experiments

We have attempted to repeat the transmission experiments made by Bruce, in Uganda, with the tse-tse fly. These flies are rather numerous in the bush along the river banks on either side of Leopoldville, and a fair number are daily brought to the laboratory by a gang of boys supplied for the purpose by the local authorities. Practically all the flies brought in by them have been *Glossina palpalis* (native name, 'mavekwa'). Unfortunately, we have had the greatest difficulty in obtaining monkeys and have only been able to use two for these experiments. Both in the Congo and in the Gambia, experiments have shown that the guinea-pig is, perhaps, the laboratory animal most susceptible to *Trypanosoma gambiense*. We, therefore, determined to employ it, lacking monkeys, in our transmission experiments. At the time of writing both experiments with monkeys remain unsuccessful, and only one, a direct transmission experiment with guinea-pigs, in which the flies were made to feed alternately on an infected and an uninfected animal, has given a positive result.

In conclusion, we wish to thank Dr. Inge Heiberg, who has been attached to the expedition by the Government of the Congo Free State, for his untiring kindness and the help he has given us in our work.

Kondolo (Case CII). Main symptoms severe and continued headache. No sleep.

Boyo Mitchel (Case XCI). Showing puffy face and lips, and drowsy dull expression. Taken six weeks before death.



Oparanga (Case CI). Showing extreme emaciation. Taken three days before death.

To follow page 45

THE CONGO FLOOR MAGGOT

THE CONGO FLOOR MAGGOT

A BLOOD-SUCKING DIPTEROUS LARVA FOUND IN THE CONGO FREE STATE

(*The First Interim Report of the Expedition of the Liverpool School of Tropical Medicine to the Congo*, 1903. *Received January*, 1904)

BY

J. EVERETT DUTTON, M.B., VICT.

WALTER MYERS FELLOW, UNIVERSITY OF LIVERPOOL

J. L. TODD, B.A., M.D., C.M., McGILL

AND

CUTHBERT CHRISTY, M.B., EDIN.

IN correspondence, during our stay at Boma, with the Rev. HOLMAN BENTLEY and Mr. SUTTON SMITH, both of the Baptist Missionary Society Corporation, we learned of the existence in the Lower Congo of what were called 'floor maggots,' which they described as 'keen blood-suckers.'

It was not, however, until camped at a place called Nkanga, on our way from Tumba to Lutete, in the cataract region of the Congo, that we had an opportunity of seeing specimens of these maggots. Here, the head man of a neighbouring village, after being questioned on the subject of native pests, collected for us during the night, a number of what appeared to us—at first sight—to be ordinary blow-fly maggots. On a closer inspection many of them were seen to contain bright red blood.

A day or two afterwards, when visiting a native village, we had the opportunity of seeing the natives collect these blood-suckers by digging with the point of a knife or scraping with a sharpened stick in the dust-filled cracks and crevices of the mud floors of their huts. We were soon able to find them ourselves as easily as the natives, and unearthed many larvae which contained bright red blood. In collecting them the natives selected those huts in which the occupants slept on floor mats, saying that where people slept on beds or raised platforms the maggots were not so numerous. They informed us, however, that those who slept in beds which were not raised more than eighteen inches from the ground were also bitten, and credited the maggot with the power of jumping to that height. In some of the huts we collected, in a short space of time, as many as twenty from only a small proportion of the floor crevices. Many were turned up from a depth of three inches. In some of the cracks, and in moist, soft earth, they were found at greater depths. There is

no doubt that these maggots feed only at night. Mr. BENTLEY told us that as many as fifty could sometimes be found beneath a single mattress, and that he had known boys to be so pestered by them that they had preferred to sit all night outside a house to sleeping within it.

In one village, Nzungu, we visited a hut, measuring eight feet by ten feet, in which seven boys were sleeping on a small mat, and in the dust beneath a bed-platform on which slept a man and a woman. In the corner of the hut was the usual small fire and a sleeping pye-dog. Although we did not see the maggots actually feeding, we collected from beneath the mats and from amongst the boys' legs some half-dozen which were filled with recently sucked blood. The natives said that the maggots dropped off at once if the limb on which they were feeding was moved. There were specimens of all sizes, ranging in length from 2 to 15 mm. amongst those brought to us, and so far we have obtained them in every village we have visited. When ready to pupate, the larva lies dormant upon the surface, changes in colour to a pinky-brown, and later becomes a dark-reddish or brownish-black, chitinous, segmented, and oblong puparium.

We have never been able to substantiate the assertion, made to us on several occasions, that the maggot is able to jump to a height of eighteen inches. We think it more probable that they reach the raised beds by crawling up either the supports or the grass wall against which the bed is usually placed. We have, however, satisfied ourselves that it feeds mainly or entirely at night, and that it probably feeds nightly since blood in varying stages of digestion, and ranging in colour from bright red to black, can often be seen in its alimentary canal.

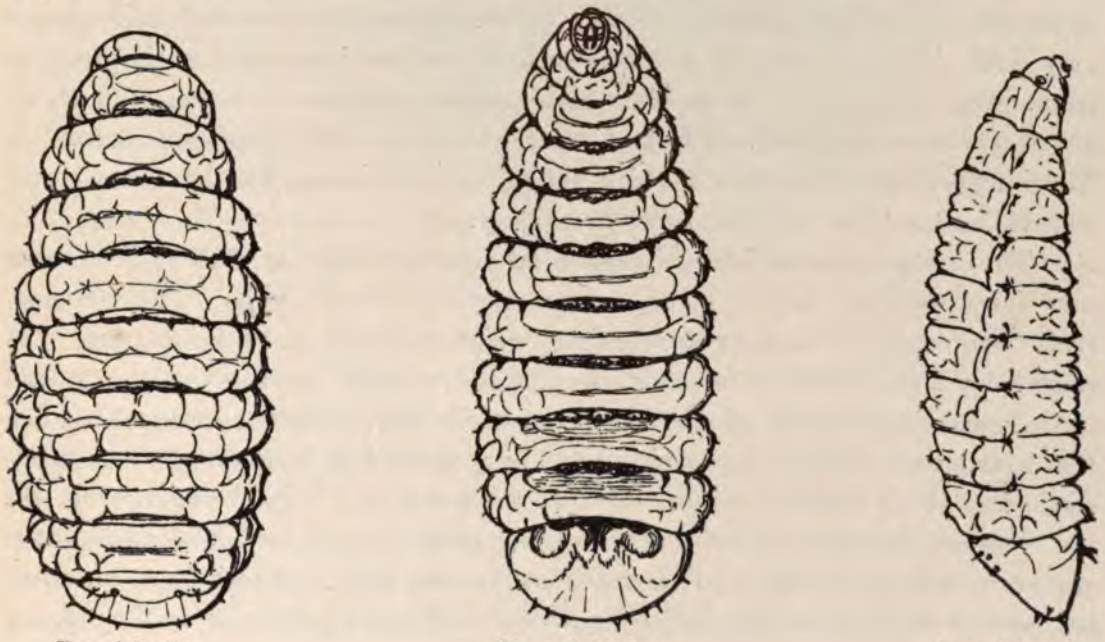
The distribution of this larva seems to be very extensive. We have collected it all over the Lutete and surrounding districts, and at Leopoldville. We have heard of it as being common at San Salvador, in Portuguese territory, on the Congo at Matadi in the cataract region, and at Tchumbiri one hundred and fifty miles above Stanley Pool.

Some of the native names for the maggot are as follows :—

At Tchumbiri, north of Leopoldville, the Bateke, according to Mrs. E. BILLINGTON of the Royal British Nurses' Association, call it 'Mabinzu.' At Leopoldville the Bateke call it 'Nchichi.' At Wathen (Lutete), in the cataract region, Mr. BENTLEY states that all maggots are called 'Ntunga,' and that there was no other special name for this one. At Matadi the native name is probably 'Mvidi.' In the Bangala district it is called 'Kiso.'

This larva maggot is semitranslucent, of a dirty white colour, acephalous, and amphipneustic. It resembles, when adult, the larvae of the bot-flies, and consists of eleven very distinct segments. The first or anterior one is divisible, by a slight constriction, into two portions, the foremost of which is small, bears the mouth parts, and is capable of protrusion and retraction to a considerable extent.

The larva is broadest at the ninth and tenth segments, is roughly ovoid in transverse section, and has, distinctly, dorsal and ventral surfaces. At the junction of the two surfaces is a row of irregular protuberances, two or more being placed on each segment. On each protuberance is a small posteriorly directed spine and a small pit. The central part of the ventral surface is flattened, and at the posterior margin of each segment is a set of three foot-pads, transversely arranged, each covered with small spines directed backwards. These aid the larva in its movements, which are fairly rapid and peculiar in that the mouth parts are protruded to the utmost and the tentacula fixed, as a purchase, first on one side then on the other, while a wave of contraction runs along the body as each segment is contracted and brought forward.



Dorsal Surface Ventral Surface Lateral Surface

CONGO FLOOR MAGGOT (× 4·5)

The last segment is larger than any of the others. Its upper surface is flattened, and looks backwards and upwards at an angle of about forty-five degrees with the longitudinal axis of the larva. This surface is roughly hexagonal and bears anteriorly, one on either side, the posterior spiracles which are seen with a pocket magnifying glass as three transverse, parallel, brown lines. Around this flattened surface, towards its border, are placed groups of rather prominent spines. The ventral surface of this segment is also flattened, and is thrown into folds by muscular contractions. The anus is situated in the anterior portion of this segment in the middle line, and is seen as a longitudinal slit, surrounded by a low ridge. Posterior to it, and on either side, is a large conspicuous spine. The anterior segment is roughly conical, and bears the mouth parts in front. Posteriorly, on the dorsal

surface, almost covered by the second segment, two spiracles, on either side, are seen with a low power as small brown spots. Two black hooks or tentacula protrude from the apex of this segment. They are curved towards the ventral surface of the maggot. The apex of each hook is blunt, and its base surrounded by a fleshy ring. Between them is the oral orifice. The tentacular processes are continued for some distance into the body of the maggot as black chitinous structures with expanded bases. There is probably, as in *Oestrus ovis* (Linn.), an articulation between the external and internal chitinous structures, since the arrangement of the mouth parts seems to be the same as in the maggot of that fly. Paired groups of minute spicular teeth are placed around the two tentacula so as to form a sort of cupping instrument. The arrangement of these teeth is as follows :—A rather large tubercle is situated on either side of and above the tentacula ; each is mounted by two or more groups of very small chitinous teeth. Just above each tentaculum is another small group of teeth. On either side of these black tentacula two irregular rows of small teeth are placed one above the other. The two latter groups are not placed upon tubercles. The integument of the larva is thick and difficult to tear. The larva is able to withstand a good deal of pressure without injury.

The following gross internal anatomical structures have at present been made out—intestinal canal, salivary gland, nervous system, and fat body. The intestinal canal commences as a short oesophagus, which ends in a proventriculus. A remarkable dorsal diverticulum, corresponding to the food reservoir of the muscid larvae, opens into the oesophagus near its anterior end. After the maggot has fed, the diverticulum is a very conspicuous object, since it is seen through the semi-transparent body wall as a bright red area when full of blood, extending from the head to about the fifth segment. The caeca, behind the proventriculus, have not as yet been well made out. The mid-intestine or chyle stomach is short, when compared with the hind intestine, and extends from the proventriculus to the junction of the urinary tubules with the gut. It lies in one or two coils. The hind intestine is very much coiled, and occupies the greatest part of the body cavity.

The urinary tubules are four in number, two on either side of the intestine. Each lateral pair combines to form a broad plate, from which is given off a single process for attachment to the gut.

Each salivary gland of the larvae consists of one very long acinus made up of large granular cells. The gland ends in a chitinous ringed duct, which joins its fellow of the opposite side to form a common duct, opening near the base of the free portion of the tentacula. The body of the maggot is lined by a white, loosely reticulated fat body. The minute anatomy of the mouth parts and sucking apparatus has not been studied.

The time required for the maturation of the larva is not yet known. The puparium is a dark-brown or black, cylindrical, segmented body, measuring 9-10·5 mm.

in length and 4-5 mm. in width. The anterior end is roughly conical, the posterior is rounded. All the external structures seen in the larva can be made out on the external surface of the puparium. The cuticle shows annular ridges.

The duration of the puparial stage is from a fortnight to three weeks.

During our stay at Wathen, Mr. BENTLEY showed us, among his collection of insects, a large light-brown fly which he believed to be developed from the floor maggots. Specimens caught in the boys' dormitory at the Wathen Mission were soon after brought in by one of our collectors, and later, while searching a native hut infested with 'floor maggots,' we saw one of these flies resting on the grass wall. Many others were subsequently found in the same building, sitting motionless amongst the beams and cob-webs of walls and roof. Because of their colour, which corresponded exactly with the smoke-stained straw and rafters of the huts, they were difficult to see, and in the dark interiors more difficult to catch.

This fly, seldom one of any other species, has since been found in many huts infested with maggots. We were told that the fly deposited its eggs on the ground of a hut, particularly in spots where urine had been voided. As a rule the fly is silent, but on one occasion we observed it buzzing loudly, fly in at the door and go directly beneath some bed mats which were raised by a low platform, some eight centimetres from the floor.

Both Mr. BENTLEY and the natives state that this fly never bites men.

The native name at Wathen for all flies is 'Nwanzi' or 'Mbwanzi.' The name for the fly which we describe is 'Nkulu Mwanzi.' The fly is thick set, and is of about the size and build of a 'blue bottle.' It is about 10-12 mm. in length, and once seen can be easily recognized. Its general colouring is tawny, but the small black hairs covering its body give it a smoky appearance. The head is large, as broad as the thorax, and protrudes in front of the eyes, which are when fresh a reddish brown in colour. The eyes are separated from each other, below, by a considerable interval and appear small in comparison with the size of the head. The proboscis is folded beneath the head in a deep groove, and is inconspicuous while in this position.

The palpi are club-shaped and covered, more particularly at their apices, with conspicuous black bristles. The third joint of the antenna is long, yellow, flattened from side to side, and rounded at its apex. It bears an arista which, thickened at its base (probably jointed), tapers to a fine point. The arista bears fine black hairs along its upper and lower borders; long at its base, the hairs become short and slanting at its apex. The dorsum of the thorax is flattened, and marked by longitudinal black and brown stripes, the transverse suture is well marked. The thorax is covered with fine black hairs and studded with rows of black bristles, which are particularly long on the sides. The squamae are very large, yellow in colour, and completely cover the yellowish-white halteres. The abdomen apparently consists of five segments. It is covered with long black hairs, and bears a few long bristles

on the last two segments. The second segment is the longest, and here the width of the abdomen is greatest, the upper surface of this segment is characteristically marked. A dark-brown or black line runs down the centre of the segment to meet at right angles a similar line which borders its posterior edge. There are no other marks on this segment, it is transparent, and its general colour is that of the rest of the body. The third segment is, except for a narrow yellow streak along its anterior border, very dark brown in colour, more marked laterally. The fourth segment is of the same dark colour, and is bordered posteriorly by a narrow lighter brown band. The fifth segment is small, and contains the genital apparatus. The wings are of a smoky brown colour, and show a well-marked venation. The legs are of the same buff as the rest of the body, and are covered with black hair and bristles. A noticeable feature of the legs is the jet-black fifth tarsal joint, which stands out prominently against the large cream-white pulvilus.

We have been able to allow a number of these maggots to feed on rats and guinea-pigs. We purpose carrying out a series of experiments with the object of determining whether they are able to play a part in the transmission of the human trypanosome. In none of the entomological works, which we can at present command, are we able to find any reference to habits or morphology by which we can identify this fly.

Specimens of the flies and larvae were submitted for identification to Mr. E. E. Austen, the dipterologist to the British Museum, together with a copy of the above report, and he has been kind enough to write as follows :—'I have read the report on the "Blood-Sucking Floor Maggot" with the greatest possible interest. Whether this maggot prove to be in any way concerned in the dissemination of trypanosomiasis or no, Messrs. Dutton, Todd, and Christy have come across an entirely novel and most interesting fact in the biology of diptera, and they are heartily to be congratulated on being the first to bring it to notice.

The flies are specimens of *Auchmeromyia luteola*, Fabr. (a species of the family Muscidae), which is widely distributed in tropical and subtropical Africa. Before reading the report, I was under the impression that the larvae of this fly were well known subcutaneous parasites of human beings and dogs, but I have now no doubt that *A. luteola* has been confused with another fly, very similar to it in appearance, but belonging to a different genus, the larvae of which are unquestionably subcutaneous parasites in man, dogs, and monkeys. Nevertheless,' says Mr. Austen, 'I have evidence, apparently reliable, which seems to show that the larvae of *A. luteola* may, perhaps, under exceptional circumstances, live subcutaneously in man. The fly itself, so far as I am aware, is otherwise harmless, and is incapable of sucking blood. Specimens in the national collection show that it ranges from Northern Nigeria to Natal ; it is therefore somewhat surprising that the habits of the larva have not been reported before.'

Fig. 1

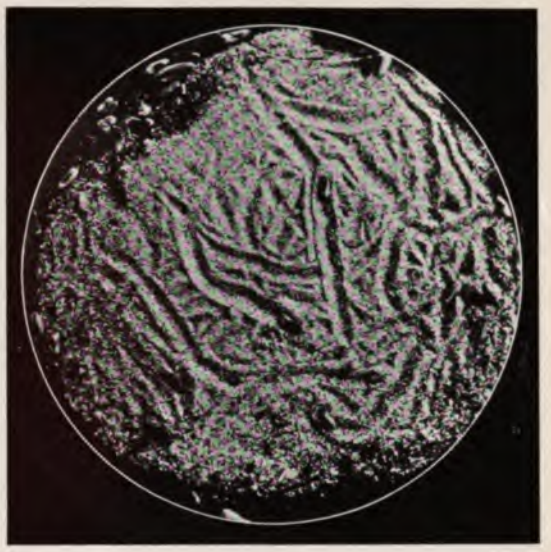


Fig. 2

Fig. 3

CONGO FLOOR MAGGOT AND FLY

Fig. 1. Flies, pupa and larvae (nat. size). Fig. 2. Markings in the sand, produced by larvae.
Fig 3. Larvae immediately after, and some hours after, feeding. (× twice).

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THE CEREBRO-SPINAL FLUID IN SLEEPING SICKNESS (TRYPANOSOMIASIS)

THE CEREBRO-SPINAL FLUID IN SLEEPING SICKNESS (TRYPANOSOMIASIS)

104 LUMBAR PUNCTURES

Second Interim Report of the Expedition of the Liverpool School of Tropical Medicine to the Congo, 1903

BY

CUTHBERT CHRISTY, M.B., C.M., EDIN.

THE majority of the punctures recorded in the accompanying tables were performed whilst working with Drs. J. E. DUTTON and J. L. TODD, in the Congo Free State. All, with the exception of three, were performed by myself either at Boma, Leopoldville, posts further up the Congo, or since returning to England. Some conclusions drawn from a number of them are published in our last conjoint report.[1]

Out of a total of sixty-four natives operated upon, the fifty-four in Table I were proved to be cases of sleeping sickness by the discovery of trypanosomes in the blood or cerebro-spinal fluid, or in hydrocele fluid ; while in the remaining ten in Table II, parasites were never found, although the majority of them were more or less suspicious cases, and all were in hospital at Leopoldville.

In thirty-four of the fifty-four sleeping sickness cases the parasites were found sooner or later in the cerebro-spinal fluid, whereas in twenty of them no parasites could be found, although in one (Case 17) the fluid was examined on five occasions.

If, however, those punctures in which the cerebro-spinal fluid was mixed with blood, and in which the parasites were found by coverslip examination to be present in the peripheral circulation on the same day as the puncture, be excluded, then we find that the result is very different, namely, forty-nine cases only, in twenty-five of which trypanosomes were found in the cerebro-spinal fluid, and twenty-four in which they were not found.

A reference to Cases 19, 20, 21, will show how important it is to exclude from all statistics those punctures in which the fluid contains trypanosomes with blood cells when the parasites are known to be in the blood stream. Cases 20 and 21 show clearly that when the fluid is mixed with blood the number of parasites appearing in it is closely in proportion, not only to the number in the blood, but to the amount of blood admitted by unskilful puncture. I therefore in this analysis will exclude from consideration all punctures in which the cerebro-spinal fluid and the blood both show trypanosomes when blood is admitted into the fluid. In the Tables these are marked with an asterisk in the name column.

1. *Second Progress Report.*

TABLE I.—SLEEPING SICKNESS CASES

+ Indicates trypanosomes present. – Indicates trypanosomes absent. C.Cms. stands for cubic centimetres.
Those marked with an asterisk are excluded from statistics owing to blood appearing in the C.S.F when trypanosomes are known to be present in the circulation. Centrif. stands for centrifugalisation.

No.	Name	Date	Sex	Age	Trypanosomes in Finger Blood	Cerebro-Spinal Fluid							Remarks on Duration of Case, Prominence of Sleep, or other Symptoms, etc., and Mode of Death
						C.Cms drawn off	Days before death	Trypanosomes	Clear or Cloudy	White Cells	Red Cells	Date of Death	
1	Elombo	17-12-03	♂	40	– 1 cover examined.	18	11	– 4 covers examined.	Clear.	..	None.	28-12-03	Duration about five months. Extreme emaciation. No symptoms of sleep. Comatose from sun exposure when punctured. Complete revival after withdrawal of fluid.
2	Lisasi .. .. *	16-12-03	♂	20	+ 5 to cover.	10	0	+ 7 to cover.	Cloudy.	..	Many.	16-12-03	Always slightly drowsy, increased before death.
3	Salamo *1	18-12-03	♀	24	+ 3 to cover.	15	2	+ 4 to cover.	Slightly cloudy.	Increased.	A few.	20-12-03	Duration about three months. Seldom seen dozing till a few days before death. Many division forms of trypanosomes in C.S.F., pericardial fluid, and bone marrow one-and-a-half hours *P.-M.*
	2	20-12-03			+ 13 to cover.	40	*P.-M.*	+ many.	Cloudy.	Increased.	None.	..	
4	Mokoko .. *	19-12-03	♂	21	+	17	..	+ 3 to cover.	Cloudy.	Increased.	Many.	..	Few symptoms. After being two months in hospital was discharged by medical officer as fit for duty. On January 5 his blood showed 1000 trypanosomes to the cover.
5	Dysiki	19-12-03	♀	26	– 2 covers examined.	18	2	+ 1 in 4 covers.	Clear.	Increased.	A few.	21-12-03	Emaciation but no sleep symptoms till day before death.
6	Bamliki	19-12-03	♂	16	– 2 covers.	6	6	– 1st centrif. 5 covers.	..	..	..	..	Extreme emaciation; slept a good deal, and was semi-comatose for three days before death. Bed-sores.
								+ 2nd centrif. 3 to cover.	Cloudy.	Scanty.	Many.	25-12-03	
7	Polulu .. *1	21-12-03	♂	21	+ 1 in 2 covers.	20	21	+ 1 in 2 covers.	Cloudy.	Scanty.	Many.	..	December 19 had severe left hemiplegic seizure. No sleep symptoms at any time. *P.-M.*—Extensive purulent meningitis. Cysticercus in triangularis.
	2	31-12-03			+ 3 to cover.		11	– 1st centrif. 2 covers.	Slightly cloudy.	Scanty.	None.	11-1-04	
								+ 2nd centrif.					
8	Tenda 1	23-12-03	♂	30	– 2 covers.	30	103	– 4 covers. 2 centrif.	Clear and limpid.	Very scanty.	None.	..	Duration about five months. Progressive emaciation. Frequently found dozing during January, but subsequently marked absence of nearly all symptoms. Retained faculties and was able to walk till hour of death. Pericardial and peritoneal fluids both showed active division forms twenty-and-a-half hours after death.
	*2	16-1-04			+ 6 to cover.	3	79	+ 1 in 2 covers.	Cloudy.	Increased.	A few.	..	
	3	27-2-04			– 2 covers.	25	37	– 4 covers. 2 centrif.	Clear and limpid.	Very scanty.	None.	..	
	4	31-3-04			– 2 covers.	16	4	+ 1 in 2 covers.	Clear.	Scanty.	A few.	4-4-04	
	5	5-4-04			– 2 covers before death.	47	*P.-M.*	– 5 covers. 13 hours *P.-M.*	Slightly cloudy.	Increased.	None.	..	

TABLE I.—*Continued*

No.	Name		Date	Sex	Age	Trypanosomes in Finger Blood	CEREBRO-SPINAL FLUID							Remarks on Duration of Case, Prominence of Sleep, or other Symptoms, etc., and Mode of Death
							C.Cms drawn off	Days before death	Trypanosomes	Clear or Cloudy	White Cells	Red Cells	Date of Death	
9	Manteka ..	1	24-12-03	♂	19	−	30	21	− 4 covers.	Clear.	Scanty.	A few.	..	Duration about three months. Rapid emaciation. Irritable and troublesome. Helpless, dazed, semi-comatose condition for two days before death.
		2	5-1-04			+	16	9	+	Clear and limpid.	Scanty.	None.	14-1-04	
10	Manga.. ..	*	26-12-03	♂	22	+	30	9	+	Slightly cloudy.	Increased.	A few.	4-1-04	Duration about five months. Marked absence of symptoms till ten days before death when sudden fit, followed by semi-comatose condition, great salivation, abolished reflexes, and erection of penis. Improved somewhat before death.
11	Kalenga ..	*1	26-12-03	♂	24	+ 1 in 2 covers.	18	45	+ 2 to cover.	Cloudy.	Scanty.	Many.	9-2-04	Marked sleep symptoms, no emaciation. Death due to sun exposure; temperature 107. Blood showed 100 trypanosomes to cover five hours before death.
		2	9-2-04			− 2 covers 1½ hours *P.-M.*	24	*P.-M.* 1½ hrs.	− 4 covers. 2 centrif.	Cloudy.	Much increased.	None.	..	
12	Maiadina ..	*1	29-12-03	♂	18	+ 2 to cover.	14	28	+ 36 to cover.	Cloudy.	Scanty.	Many.	..	Duration about four months. Had epileptic attacks, few sleep symptoms, simple-minded condition. Six days before death, tremors of limbs, opisthotonos and semi-coma. *P.-M.* Pneumonia and purulent meningitis.
		2	22-1-04			− 2 covers.	18	4	− 3 covers. 2 centrif.	Clear and limpid.	Scanty.	None.	26-1-04	
13	Kabali	1	29-12-03	♀	27	− 2 covers.	5	70	− 8 covers. 2 centrif.	Cloudy.	Increased.	A few.	..	Duration about five months. Progressive weakness, drowsiness, and emaciation from the first. No marked nervous symptoms. Bed-sores.
		2	20-2-04			+ 6 to cover.	32	17	− 5 covers. 2 centrif.	Clear.	Scanty.	A few.	..	
		3	7-3-04			− 2 covers.	15	1	− 5 covers. 2 centrif.	Clear.	Scanty.	None.	8-3-04	
14	Molumba ..	1	7-1-04	♂	32	− 2 covers.	14	..	− 5 covers. 2 centrif.	Cloudy.	Increased.	Many.	..	No marked symptoms, no drowsiness or dulness. Recurring attacks of dysentery.
		2	5-2-04			− 1 in 2 covers.	22	..	− 3 covers. 2 centrif.	Clear and limpid.	Scanty.	None.	..	
15	Kondolo ..	1	8-1-04	♂	32	− 2 covers.	20	80	− 5 covers. 3 centrif.	Clear and limpid.	Very scanty.	None.	..	Three months in hospital. Marked absence of symptoms except headache. Ten days before death headache severe. Suicide by drowning.
		2	14-3-04			− 2 covers.	22	14	− 2 covers. 2 centrif.	Clear and limpid.	Very scanty.	None.	28-3-04	
16	Ejoli	..	7-1-04	♂	28	− 2 covers.	8	19	− 3 covers. 3 centrif.	Clear.	Increased.	None.	..	Drowsiness. Great emaciation. Few symptoms. Conscious till hour of death.

TABLE I.—*Continued*

No.	Name	Date	Sex	Age	Trypanosomes in Finger Blood	Cerebro-Spinal Fluid							Remarks on Duration of Case, Prominence of Sleep or other Symptoms, etc., and Mode of Death
						C.Cms drawn off	Days before death	Trypanosomes	Clear or Cloudy	White Cells	Red Cells	Date of Death	
17	Oparanga .. 1	7-1-04	♂	18	– 2 covers.	16	62	– 3 covers. 2 centrif.	Clear and limp.	Scanty.	None.	..	Duration five or six months. Progressive weakness and emaciation from the beginning. Extreme emaciation at death. No dulness or marked symptom at any time, except drowsiness during January, but less subsequently. Could walk, and retained faculties till hour of death. Trypanosomes in blood scanty and seldom seen.
	2	27-1-04			+ 3 to cover.	20	42	– 7 covers. 3 centrif.	Clear and limp.	Scanty.	A few.	..	
	3	11-2-04			– 2 covers.	20	27	– 2 covers. 2 centrif.	Clear and limp.	Scanty.	None.	..	
	4	23-2-04			– 2 covers.	14	15	– 3 covers. 2 centrif.	Clear and limp.	Scanty.	None.	..	
	5	2-3-04			+ 48 to cover.	10	7	– 6 covers. 2 centrif.	Clear and limp.	Scanty.	None.	9-3-04	
18	Kimfuta	15-1-04	♀	9	+ 8 to cover.	16	5	+ many.	Clear and limp.	Very scanty.	None.	20-1-04	Duration about two months only. Rapid emaciation, weakness, tremors, and vacancy. Sleep symptoms only present towards the end.
19	Banja *1	20-1-04	♂	26	+ 80 to cover.	8	..	+ many.	..	..	Much blood.	..	Was admitted January 5 from prison, chained to two other prisoners, and charged with cannibalism. Emaciation due to starvation. By end of February quite well and putting on flesh. At end of April started for England, arriving apparently in robust health. Now fat and well, and is employed in Johnston Laboratory, Liverpool.
	2	8-2-04			+ 100 to cover.	50	..	– 4 covers. 2 centrif.	Clear and limp.	Scanty.	None.	..	
	3	26-2-04			+ 32 to cover.	20	..	– 4 covers. 2 centrif.	Clear and limp.	Scanty.	None.	..	
20	Ieri 1	20-1-04	♂	11	+ 2 to cover.	20	19	+ 1 in 2 covers.	Clear and limp.	Very scanty.	None.	..	Duration about four months. Progressive weakening and wasting interrupted by periods of partial recovery. Somnolence marked towards the close. Appetite voracious to within three days of death. Marked retraction of head and opisthotonos for several days before death. *P.-M.*—No signs of purulent meningitis.
	2	27-1-04			+ 4 to cover.	10	12	+ 4 to cover.	Clear and limp.	Very scanty.	None.	..	
	*3	5-2-04			+ 150 to cover.	10	3	+ 40 to cover.	Cloudy.	Scanty.	Many.	..	
	*4	8-2-04			+ 250 to cover.	15	0	+ 1 in 2 covers.	Clear.	Scanty.	A few.	8-2-04	
21	Moldi 1	21-1-04	♀	24	– 2 covers.	6	16	– 3 covers. 2 centrif.	Clear.	Scanty.	A few.	..	Duration about three months. Drowsiness a marked symptom latterly. Very little wasting. Sensations much dulled. Lumbar puncture without cocaine.
	*2	25-1-04			+ 13 to cover.	35	12	+ 3 in 4 covers.	Clear.	Scanty.	A few.	..	
	*3	5-2-04			+ 100 to cover.	15	1	+ 20 to cover.	Slightly cloudy.	Scanty.	Many.	6-2-04	
22	Kapinga	23-1-04	♀	24	+ 1 in 2 covers.	20	12	+ 1 in 3 covers.	Slightly cloudy.	Much increased.	None.	4-2-04	No drowsiness at any time. Progressive weakness, vacancy, and emaciation.

TABLE I.—*Continued*

No.	Name		Date	Sex	Age	Trypanosomes in Finger Blood	CEREBRO-SPINAL FLUID							Remarks on Duration of Cases, Prominence of Sleep and other Symptoms, etc., and Mode of Death
							C.Cms drawn off	Days before Death	Trypanosomes	Clear or Cloudy	White Cells	Red Cells	Date of Death	
23	Patesa		3-2-04	♀	12	+ thousands, 15 to field.	10	0	– 6 covers. 2 centrif.	Clear.	Scanty.	A few.	3-2-04	Only four days under observation. Admitted for dysentery. Emaciation extreme. No drowsiness or vacancy. L.P. four hours before death. Spleen punctured at same time showed 80 trypanosomes to field.
24	Boyo		9-2-04	♂	19	– 2 covers.	32	0	– 3 covers. 2 centrif.	Slightly cloudy.	Increased.	None.	9-2-04	Duration three to four months. L.P. soon after convulsive seizure, with high temperature and unconsciousness, due to sun exposure. Six hours before puncture, finger blood showed 100 trypanosomes to the cover.
25	Benjamin		9-2-04	♂	28	+ 2 to cover.	13	1	+ 9 to cover.	Slightly cloudy.	Greatly increased. Early pus.	None.	10-2-04	Duration about three months. Progressive weakness and emaciation. Seldom drowsy. Tremors almost amounting to rigors before death, which was hastened by sun exposure. *P.M.*—Intense cerebral congestion.
26	Boyo Mitchel	1	11-2-04	♂	16	– 1 cover.	18	103	+ 3 to cover.	Cloudy.	Increased.	Many.	..	Duration about five months. Drowsiness very marked. Extreme emaciation during last month on voyage to England. Died on the way to Liverpool.
		2	27-2-04			– 2 covers.	16	99	– 4 covers. 2 centrif.	Clear.	Increased.	None.	..	
		3	31-3-04			– 2 covers.	25	54	+ 1 in 2nd centrif.	Slightly cloudy.	Increased.	Many.	..	
		4	23-5-04			+ 5 to cover.	35	1	+ 1 in 5 covers.	Clear and limp.	Scanty.	None.	24-5-04	
		5	24-5-04			– 1 cover.	30	*P.-M.*	–	Cloudy.	Increased.	None.	..	
27	Kabongo		13-2-04	♂	35	+ 4 to cover.	4	..	– 4 covers. 2 centrif.	Clear and limp.	Scanty.	None.	..	Very few symptoms. No drowsiness. Still under observation. On March 21, hydrocele fluid negative, three covers examined; blood positive, 12 to cover.
28	Kikoffi	1	20-2-04	♂	19	+ 10 to cover.	30	..	+ 60 to cover.	Slightly cloudy.	Much increased.	None.	..	Still under observation. Nervous tremors and exaggerated reflexes on admission, but improved subsequently.
		2	25-3-04			– 2 covers.	26	..	+ 1 in 1 cover.	Clear and limp.	Scanty.	None.	..	
29	Yaiyai	1	24-2-04	♀	20	– 2 covers.	42	9	– 3 covers. 2 centrif.	Slightly cloudy.	Increased.	A few.	..	Duration two months only. Rapid progressive weakness and wasting. Symptoms of drowsiness not marked. Extensive bed-sores. Conscious till hour of death.
		2	28-2-04			– 4 covers.	10	7	+ 1 in 3 covers.	Clear.	Increased.	None.	..	
		3	4-3-04			– 2 covers.	15	0	– 4 covers.	Clear and limp.	Scanty.	None.	4-3-04	

TABLE I.—*Continued*

No.	Name		Date	Sex	Age	Trypanosomes in Finger Blood	CEREBRO-SPINAL FLUID						Remarks on Duration of Cases, Prominence of Sleep and other Symptoms, etc., and Mode of Death
							C.Cms drawn off	Days before Death	Trypanosomes	Clear or Cloudy	White Cells	Red Cells	Date of Death	
30	Plekal		26-2-04	♂	27	– 1 cover.	22	9	– 5 covers.	Clear and limp.	Scanty.	None.	4-3-04	Admitted February 2 for dysentery. No drowsiness. Great emaciation.
31	Bugwendi		2-3-04	♂	16	+ 48 to cover.	25	19	– 5 covers. 2 centrif.	Clear and limp.	Scanty.	None.	..	Duration about three months. Irritable, hysterical, and troublesome. March 16, wasting and incoordination of movements. March 20, suddenly drank his urine from pot. Then developed incoherent jabber, spasmodic contraction of arms, with athetosic movements of fingers, and picking at blanket. *P.-M.*—Lymph round cord, ventricles of brain distended, etc.
			21-3-04			– 2 covers.	15	0	– 3 covers. 2 centrif.	Slightly cloudy. Straw colour.	Scanty.	Many.	21-3-04	
32	Belambo ..	1	27-2-04	♂	26	– 4 covers.	24	46	+ 1 to 4 covers.	Cloudy.	Greatly increased.	Many.	..	Duration about three months. Drowsiness at no time very marked, but nervous symptoms, tremors, and increased reflexes from day of admission. On March 19 developed spasmodic flexion of arms with convulsive movements of face and many muscles of body. No wasting. Appetite good, and conscious till hour of death. Reflexes nearly absent at death.
		2	9-3-04			..	18	35	+ 1 in 4 covers.	Clear.	Increased.	None.	..	
		3	30-3-04			– 2 covers.	20	14	+ 80 to cover.	Cloudy.	Increased.	Many.	..	
		4	13-4-04			– 2 covers.	40	0	+ 1 in 6 covers.	Clear and limp.	Scanty.	None.	13-4-04	
33	Litali		29-2-04	♂	26	– 5 covers.	18	2	+ 3 to cover.	Slightly cloudy.	Greatly increased.	None.	2-3-04	Admitted to hospital as a jabbering lunatic. Not suspected of sleeping sickness. Under observation for two months. Trypanosomes never found in blood. Slept a good deal latterly.
34	Mozao	1	11-3-04	♂	16	+ 3 to cover.	18	7	– 5 covers. 2 centrif.	Clear and limp.	Scanty.	None.	..	No very marked symptoms, but extreme emaciation.
		2	18-3-04			– 2 covers.	4	0	– 3 covers.	Clear.	Increased.	None.	18-3-04	
35	Batanga		14-3-04	♂	27	– 2 covers.	24	24	+ 2 to cover.	Clear.	Slightly increased.	None.	7-4-04	Duration about three months. Marked sleep symptoms. Epileptic attack March 17. At L.P. pressure of fluid considerable. Improvement in symptoms after puncture.
36	Bandela ..	1	16-3-04	♂	10	– 2 covers.	22	30	– 4 covers. 2 centrif.	Clear.	Increased.	None.	16-4-04	Duration five months. March 12, had appearance of bloated cretin, with marked sleep symptoms, which disappeared for several days after L.P. Salivation, no wasting. *P.-M.*—Large quantity ventricular and subarachnoid fluid.
		2	16-4-04			– 1 cover from heart.	50	*P.-M.*	– 3 covers. 8 hours' *P.-M.*	Cloudy.	Much increased.	None.	..	

TABLE I.—*Continued*

No.	Name	Date	Sex	Age	Trypanosomes in Finger Blood	CEREBRO-SPINAL FLUID						Remarks on Duration of Cases, Prominence of Sleep and other Symptoms, etc., and Mode of Death
						C.Cms drawn off	Days before Death	Trypanosomes	Clear or Cloudy	White Cells	Red Cells	Date of Death	
37	Mengo..	10-3-04	♂	24	– 2 covers.	26	..	– 6 covers. 2 centrif.	Clear.	Scanty.	A few.	..	Admitted March 2 with very marked nervous symptoms; childish and irritable. One flick with finger on patella caused uncontrollable clonic movements of legs, and a light tap caused violent extension of whole body and legs, so powerful as to throw him off a chair yet able to walk about and do light work. Observations up to April 29, 1904. * Refers to hydrocele fluid only.
	Hydrocele fluid	10-3-04			– 2 covers.	..	..	+ 14 to cover.	Cloudy.	Large quantity.	None.	*	
	" "	25-3-04			– 2 covers.	..	..	+ 2 in half deposit.	Cloudy.	Large quantity.	None.	*	
38	Tumba .. 1	26-3-04	♂	21	– 2 covers.	35	4	+ 20 to cover.	Slightly cloudy.	Scanty.	Many.	..	Duration about three months. Marked dulness and sleep symptoms. March 22, developed tremors and flexions of arms, which increased after L.P. on 26th. Conscious up to time of death.
	2	30-3-04			– 2 covers.	34	0	– 4 covers. 2 centrif.	Clear and limp.	Scanty.	None.	30-3-04	
39	Molar	26-3-04	♂	14	– 2 covers.	15	..	+ 2 covers.	Clear.	Scanty.	A few.	..	Mission boy, only under observation for a few days.
40	Mokindi	2-4-04	♂	18	+ 4 to cover.	20	..	– 5 covers. 2 centrif.	Clear and limp.	Very scanty.	None.	..	An early case. Did not admit that he was sick. Marked dulness, but no sleep. Distension of belly.
41	Nakunyi	4-4-04	♂	17	+ 16 to cover.	18	4	– 4 covers. 2 centrif.	Clear and limp.	Very scanty	None.	8-4-04	Admitted a month before death for dysentery. Great emaciation and weakness, otherwise few symptoms, and none of sleep.
42	Pania	6-4-04	♂	18	+ 4 to cover.	18	..	– 5 covers. 2 centrif.	Clear and limp.	Scanty.	None.	..	An early case. Few marked symptoms and none of sleep.
43	Kitambala	7-4-04	♂	13	– 4 covers.	18	..	– 4 covers.	Clear and limp.	Scanty.	None.	..	An early case. Marked dulness, but no sleep.
44	Katambue	14-4-04	♂	24	– 2 covers.	26	..	+ 300 to cover.	Slightly cloudy.	Increased.	A few.	..	An advanced case. Only under observation for one day. Was doing duty up to February 27, 1904.
45	Kabela	14-4-04	♂	26	– 2 covers.	20	..	+ 16 to cover.	Slightly cloudy.	Scanty.	A few.	..	An advanced case. Only under observation for one day. Was doing duty up to February 17, 1904.
46	Ekongo	18-4-04	♂	21	– 2 covers.	22	..	+ 6 to cover.	Slightly cloudy.	Increased.	A few.	..	Advanced case seen on the march, and punctured under difficulties in the grass.
47	Mbeka..	18-4-04	♂	19	+ 2 to cover.	20	..	– 4 covers. 2 centrif.	Clear and limp.	Scanty.	None.	..	An early case, with no symptoms.

TABLE I.—*Continued*

No.	Name	Date	Sex	Age	Trypanosomes in Finger Blood	CEREBRO-SPINAL FLUID							Remarks on Duration of Cases, Prominence of Sleep, or other Symptoms, etc., and Mode of Death
						C.Cms. drawn off	Days before death	Trypanosomes	Clear or Cloudy	White Cells	Red Cells	Date of Death	
48	Kitambue	23-5-04	♂	24	+ 4 to cover.	18	17	+ 1 in 4 covers.	Slightly cloudy.	Much increased.	None.	9-6-04	Admitted to hospital beginning of March. Started for England April 29. Weakness increased during voyage and sleep a marked symptom. Admitted Royal Infirmary, Liverpool, May 24. Slept almost continuously before death.
49	Tomi	23-5-04	♂	17	+ 5 to cover.	4	25	− 3 covers. 2 centrif.	Clear and limp.	Slightly increased.	None.	..	Boy from Kinshassa Mission, Leopoldville. Duration of case about five months. No sleep symptoms. Progressive wasting and weakness.
		15-6-04			+	30	1	+ many. 5 to field.	Clear.	Increased.	None.	17-6-04	
		17-6-04			+	45	*P.-M.*	− 2 covers.	Slightly cloudy.	Much increased.	None.	..	
50	Nbela	9-10-03	♂	24	..	35	23	+ many.	Slightly cloudy.	Much increased.	None.	1-11-04	An advanced case seen at Boma. Marked somnolence. Admitted August 1.
51	John Paka	26-10-03	♂	28	+ 2 to cover.	10	..	− 6 covers.	Clear and limpid.	Very scanty.	A few.	..	An early case. A Sierre Leone boy seen at Boma. No symptoms and no complaint.
52	Louis .. .. *	16-10-03	♂	16	+ 11 to cover.	15	..	+	Slightly cloudy.	Increased.	A few.	Died.	An advanced case in hospital at Boma.
53	Somi	13-10-03	♀	22	+ 6 to cover.	20	..	+ many.	Slightly cloudy.	Increased.	None.	Died.	An advanced case seen in hospital at Boma.
54	Bulumbu	29-10-03	♀	16	− 1 cover.	10	..	+	..	..	..	Died.	An advanced case seen at Boma.

TABLE II.—DOUBTFUL CASES NOT PROVED TO BE SLEEPING SICKNESS

No.	Name	Date	Sex	Age	Trypanosomes in Finger Blood	Cerebro-Spinal Fluid							Remarks on Duration of Case, Prominence of Sleep, or other Symptoms, etc., and Mode of Death
						C.Cms. drawn off	Days before death	Trypanosomes	Clear or Cloudy	White Cells	Red Cells	Date of Death	
1	Mwandu	22-12-03	♂	21	– 2 covers.	20	8	– 6 covers.	Clear.	Scanty.	A few.	31-12-03	Apparently a case of miliary tubercle. No symptoms of sleeping sickness.
2	Lembi	24-12-03	♂	23	–	5	9	– 5 covers.	Clear.	Scanty.	A few.	2-1-04	Probably a sleeping sickness case. Extreme emaciation.
3	Salabantu	30-12-03	♂	19	–	15	..	– 4 covers.	Clear and limpid.	Scanty.	None.	..	Discharged as fit. No symptoms of sleeping sickness.
4	Saoka	7-1-04	♂	17	–	10	..	– 3 covers. 2 centrif.	Clear and limpid.	Very scanty.	None.	..	A doubtful case, with symptoms of dulness and childishness, admitted December 26 and discharged as fit March 23, 1904.
5	Luisi	10-1-04	♀	24	–	14	0	– 5 covers.	Clear and limpid.	Scanty.	None.	10-1-04	A doubtful case, with marked emaciation and some sleep symptoms.
6	Kanyinki	22-1-04	♂	24	–	18	33	– 3 covers. 3 centrif.	Clear and limpid.	Scanty.	None.	25-2-04	A doubtful case, admitted for severe dysentery. Extreme emaciation but no dulness or drowsiness.
7	Sungula	5-2-04	♂	30	–	40	0	– 4 covers. 2 centrif.	Milky.	Large deposit of pus.	None.	5-2-04	Doubtful history of sleeping sickness; no emaciation. Retraction of head and convulsive movements of arms. Died a few hours after admission. *P.-M.*—Purulent meningitis and ethmoidal sinuses full of pus.
8	Fatiala	8-2-04	♀	20	– 2 covers.	22	4	– 3 covers. 2 centrif.	Clear.	Increased.	A few.	12-2-04	A septic case, with large collection of pus in arm when admitted.
9	Pongor	10-2-04	♂	24	– 3 covers.	15	0	– 1 cover.	Greenish-yellow cloudy fluid.	Pus.	None.	10-2-04	Semi-comatose when admitted. Somewhat similar case to No. 8. Excessive continuous rigors, and pus oozing from nostrils. Violent spasmodic contraction of limbs.
10	Kabinda	27-2-04	♂	18	– 1 cover.	25	..	– 2 covers. 2 centrif.	Clear and limpid.	Very scanty, almost none.	None.	..	Only in hospital half-an-hour asking for treatment for craw-craw.

In studying the column in Table I, in which is enumerated the number of days intervening between the puncture and the date of death, it will be seen that the probability of trypanosomes being found in the cerebro-spinal fluid tends to increase as one nears the fatal termination. This is shown by the following two Tables compiled from the forty-nine cases under analysis.

TABLE III

Thirty-five cases known to have proved fatal at the date of compilation of Table I

Days before Death	Number of Punctures	Trypanosomes present	Trypanosomes absent
Within ten days	27	14	13
Between ten and thirty	19	8	11
Between thirty and one hundred and ten ...	11	4	7
Totals	57	26	31

TABLE IV

Fourteen cases not known to be fatal at the time of compilation of Table I

Days before Death	No. of Punctures	Trypanosomes present	Trypanosomes absent
The majority are comparatively early cases, and presumably many days from death	17	6	11

A larger series of lumbar punctures in the earlier stages would be of the greatest interest.

In the Congo disease, as we have seen it, mainly at Leopoldville, there is an endless variety of types. In most cases sleep is absent, in some dulness and apathy are prominent, in others nervous symptoms and mania are conspicuous, while a proportion of cases have only progressive emaciation and fever. In classifying these symptoms it does not seem possible to definitely connect them with the appearance or non-appearance of trypanosomes in the cerebro-spinal fluid.

Cases 8 and 17, each of them punctured several times with negative results, except in one instance, were conspicuous for general absence of all symptoms except fever and emaciation. They each were able to walk and retain their faculties up to the time of death. Case 13 is in many respects similar to the foregoing two, but developed increased drowsiness some time before death, although showing no parasites in the cerebro-spinal fluid.

or white cells, or both, I have here described it as clear, slightly cloudy, or cloudy. If with blood only the cloudiness has a pink tinge, but if with white cells only it has no pink tinge. It is invariably clouded almost immediately after death by excess of white cells. The colour and extent of the cloudiness is best gauged by looking down and not through the centrifuge tube.

The fluid, as soon as drawn, should be centrifugalized gently for fully five minutes. It can then be poured off to the last drop into another tube, leaving only the resulting small deposit, which can then be picked up with a fine pipette, placed on a slide and systematically examined under a coverglass well ringed with vaseline, with a one-eighth or one-sixth objective, and a No. 4 eyepiece. If centrifugalized violently for a length of time the activity of the trypanosomes may be much decreased, and, consequently, the labour of searching for them more difficult, and if there is much deposit they may even be mutilated by the pressure of the cells. I have found it far better to centrifugalize the fluid a second time after the deposit from the first centrifugalization has been examined, for, owing to degeneration changes, which commence in the cells almost as soon as the fluid is drawn, it is best to examine it and fix some films without delay. It is imperative that the fluid should be centrifugalized a second or even a third time if possible, for, on two occasions at least, I have found trypanosomes in the second deposit and not in the first. If there is no deposit in the tube the lowest fluid should be pipetted without pouring off, for the parasites are easily poured away with the fluid.

With regard to the white cell elements, I have described them as very scanty, scanty, increased, and much increased, and I have assumed that very scanty is the normal condition. The difference between these various degrees is difficult to make out, and is apt to depend upon the amount of fluid allowed to remain with the deposit.

TABLE V

L.P.	Very Scanty	Scanty	Increased	Much Increased	Totals
Tryps. present	3	10	11	7	31
Tryps. absent	7	25	10	1	43
Total ...	10	35	21	8	74

The majority of the punctures come under the headings of scanty or increased, viz., forty-six per cent. under the former, and twenty-six per cent. under the latter, leaving about thirteen per cent. for both very scanty and much increased.

With regard to anaesthetics, cocaine is the best. Cases of excitement or mania necessitate chloroform, but it is inadvisable to give chloroform if it can be avoided, for, in two cases, the struggle and subsequent exhaustion have I believe hastened a fatal issue.

The needle selected should be as fine as possible, consistent with sufficient strength to withstand the grip of the powerful back muscles, if in the early stages of the disease the patient is restless or insufficiently cocainised. In length it should be from two to three inches. It is important that the diagonal surface at the point should be as short as possible, and the actual point not too sharp. In no case should the syringe be used as a handle for the needle, but into the base of the needle should be screwed a metal handle, which, when the needle has been passed into position, can be substituted for a glass 10 c.c. serum syringe with a short rubber connexion. The careful sterilization of all instruments is of course necessary. Although the skin may be cleansed as thoroughly as possible, it cannot be sterilized, but, in my cases, no introduction of septic matter has resulted from the operation. It would, however, probably be wisest, after the cocaine has been injected, to cauterize the site of the puncture with some small cautery made for the purpose.

In the cases of septic meningitis, pus is frequently found in the ethmoidal or other sinuses, and the infection cannot be traced to the lumbar puncture. In Cases 7 and 9, in Table II, and others in Table I, symptoms of meningitis were apparent before the puncture was made, and in other cases death occurred from the same cause without lumbar puncture. The discovery of purulent lymph round the cord in Case 31 might possibly point to infection introduced with the needle, but in this case the symptoms did not commence till a fortnight after the puncture.

These notes are the outcome solely of work done in connexion with the Congo disease.

The duration of the cases in Tables I and II is gauged chiefly by the date of the commencement of symptoms, that is in many cases the date at which the patient finds himself unable to work, the actual duration probably being much longer. The disease can frequently be diagnosed by the intimate friends or fellow workmen of the patient long before he himself realizes it.

The total number of cases here recorded is too few to permit of any very definite statements, but I think it is sufficient to allow of the following provisional conclusions.

1. That in many cases the trypanosomes never find their way into the cerebro-spinal fluid, and in those cases in which they do they are more likely to be found towards the termination of the disease.

2. That the commencement of the fever or other symptoms is in no way co-related to the entrance of the parasites to the cerebro-spinal fluid.

3. That a large number of trypanosomes in the cerebro-spinal fluid is rare, but when it does occur there is usually an access of temperature.

we believe that a definite increase of virulence, as well as an attenuation, can be achieved, the former by the passing of the trypanosomes through a large series of animals of the same species, and the latter by constantly changing the species of animal employed.

Rats.—Young rats, thirty experiments, shortest incubation two days, longest thirty-five, average from four-and-a-half to seven-and-a-half days. Usual duration from twenty to forty-one days. Some have survived one hundred and thirty-two days. Adult rats, thirty-three experiments, incubation from four to forty-seven days, average eight-and-a-half days. Duration from forty-five to three hundred and eighty-eight days. Some rats inoculated in Senegambia in November, 1902, still survive, but their blood is negative microscopically, and non-infective. The parasites are present in the blood of both young and adult rats in fair quantities, though in many of the animals they are not constantly present, being at one time numerous in the blood, and a few days later absent altogether, or only demonstrated after a most careful search.

Mice.—Twenty-five experiments. Incubation period from one to thirty-seven days; average from four to seven days. Usual duration from eleven to fourteen days. In some cases the parasites have been very numerous, and continually present in the blood, but very often the animal shows only a few parasites, or there may be a marked irregularity as to the appearance and disappearance of the organism.

Guinea-pigs.—Ten experiments. Three guinea-pigs have become infected after twelve, fifty-three, and seventy-three days, respectively, and have lived three, four, and eight months. One, Experiment 114,[1] inoculated May 13 and positive twelve days later, never showed parasites again in its peripheral blood until September 11. On November 1 thirty to a field[2] were observed. The trypanosomes continued to increase in numbers, and on December 3 reached eighty to one hundred to a field. On December 20 the numbers decreased and death ensued on January 4. A guinea-pig, Experiment 133,[3] inoculated on July 20 from a rat, was positive on September 11, parasites became very numerous on November 1 (twenty to field), and continued so to the end on November 20. Rupture of the spleen was the cause of death. Four guinea-pigs, inoculated one hundred and twenty-four days ago with large quantities of blood containing numerous trypanosomes, have remained negative up to the present time, despite re-inoculations. Their controls—*i.e.*, rats and rabbits—became infected in the usual time. Three guinea-pigs, inoculated on March 7 from rat 279A with large doses of blood containing countless trypanosomes, showed parasites in their blood in twelve hours in two cases, and in three days in the other. The

1. J. E. Dutton and J. L. Todd, *First Report of the Trypanosomiasis Expedition to Senegambia* (1902), Liverpool School of Tropical Medicine. Memoir XI.

2. We have, throughout, used three-quarter inch square cover-slips, and examined with one-sixth inch objective, and No. II eye-piece.

3. Dutton and Todd, *loc. cit.*

parasites were seen in their blood for a few days (about twenty-four to a cover). Since March 20 these animals have been negative.

Rabbits.—Incubation period from five to fifteen days; average from seven to nine days. Duration from fifty to one hundred and twenty-eight days. In some cases the organisms have been fairly constant in the peripheral blood, in others they appear and disappear at irregular intervals, and the numbers vary from a few to twenty to a field. In these animals loss of weight and diminution of red cells and haemoglobin are constant features of the disease. In only one rabbit, which lived one hundred and twenty-eight days, have we noticed a purulent discharge from the nose, eyes, etc., with loss of hair, and then only in the last six weeks of its existence.

Cats.—Two experiments, both inoculated intravenously. Experiment 422, inoculated March 12 from a mouse. Trypanosomes were observed on March 19. Parasites were constantly present, the numbers usually being small. The temperature rose on March 20 to 104·4° F. and on the 22nd to 104·6°. Some loss of weight and anaemia have been observed. Experiment 447, inoculated March 22 from a rat; positive on March 26. Parasites were constantly present and in fair numbers—often one to each field. No rise of temperature was observed.

Puppies (from three to seven months).—Three experiments. Incubation periods, five days after intravenous and ten days after intraperitoneal and subcutaneous inoculation. Duration from thirty-three to forty-three days. In two cases the parasites were to be found almost constantly and in fair numbers which increased in the later stages of the disease; before death the blood became negative again. In the remaining experiment trypanosomes were only to be found at intervals and in small numbers. Loss of weight occurred in all three, and in two cases there was a marked diminution in the number of red corpuscles with a corresponding decrease in the percentage of haemoglobin. Subnormal temperature was recorded in one case before death.

Goat.—Small female, Experiment 518. Inoculated intraperitoneally on April 14 from a rat showing numerous trypanosomes. Strain 'Lammin.' Parasites appeared in the blood on April 21, but have not been found since. No rise of temperature nor other symptoms have yet been observed.

Donkey.—Inoculated subcutaneously on February 23 from a rat. Positive on March 9. Parasites were found on rare occasions, and then only in very small numbers. There was no definite rise of temperature. Some diminution in the percentage of haemoglobin and the number of red cells has been observed.

Monkeys.—Macacus rhesus, Experiment 311. This monkey had been infected by Dr. H. E. ANNETT with blood from the European Case H. K.[1] Parasites were found only at intervals and in small numbers, though the animal appeared to be very

1. H. E. Annett, *First Report of the Trypanosomiasis Expedition to Senegambia*, 1902, Liverpool School of Tropical Medicine, Memoir XI, p. 4.

ill and emaciated ; at the end of nine months it had quite recovered. In January, 1904, rats inoculated from it during the preceding four months not having become infected we decided to attempt to reinfect the animal. On January 29, thirteen months after its original infection, it was inoculated with blood from a rabbit ('Gunjur' strain). On February 6 parasites were found in the blood ; they were almost always present, though not in great numbers, up to March 24, from which date until death, on April 3, the blood remained negative. Very marked oedema of both upper and lower eyelids, intraorbital space, and eyebrows occurred on March 27 ; this persisted for three days ; no parasites were to be found in the oedema fluid. No other symptoms other than loss of weight and anaemia were observed.

Macacus rhesus, Experiment 517. Inoculated intraperitoneally on April 14 from a rat showing numerous trypanosomes. Strain 'Lammin.' Parasites appeared in the blood on April 16. Death occurred on April 18 from purulent and haemorrhagic peritonitis caused by a parasite found in the intestinal walls and not yet identified.

A chimpanzee, Experiment 134,[1] inoculated by DUTTON and TODD in July, 1903. Parasites were rarely seen in the blood, and only in small numbers. After the third week in September trypanosomes were found only once in the blood, on January 27, when one was seen. On this date the temperature rose from its usual level of between 99° and 100° to 103·6° F., falling again next day. Parasites were not seen again before death, which occurred from broncho-pneumonia on February 10.

Sub-inoculations.—Two mice and a rat inoculated on November 20 did not become infected. Of the two mice and a rat inoculated on November 27 the mice did not become infected, while the rat was positive once, January 7, when one trypanosome was found in its blood. Two mice and a rat inoculated with large doses of heart blood immedately after death never became infected.

Symptoms.—There were occasional rises of temperature to 102° to 103° F. with no apparent cause. One of these occasions (November 27) one trypanosome was found in the blood. There were temporary loss of weight and appetite and falling off in condition towards the end of December, 1903. The animal subsequently improved and regained appetite. A blood count in July, 1903, showed haemoglobin 85 per cent. and red cells 8,360,000 per cubic millimetre. One made on February 6, 1904, showed haemoglobin 60 per cent. and red cells 4,540,000 per cubic millimetre.

Horse.—Experiment 87. The small bay stallion[2] inoculated in West Africa by DUTTON and TODD in February, 1903, and brought to England in July, 1903, is alive and in good condition, and seems to have recovered. Trypanosomes have not been seen in its blood since it was brought to England. Two rats inoculated from it at the end of July became infected. Rats and mice inoculated on October 2 and

1. Dutton and Todd, *loc. cit.*
2. Dutton and Todd, *loc. cit.*

Goat.—One experiment, a female. Incubation period, nine days. Parasites were fairly constant in the blood during the first week of infection, but afterwards were found only rarely. There was no rise of temperature with the appearance of parasites in the blood, but five days later, when eight trypanosomes were seen to the cover, the temperature rose to 104° F. No other symptoms have as yet been observed.

Donkey.—Inoculated on March 28 subcutaneously from a rat. On April 7 the temperature rose to 102·7° F., and on the 9th to 103·2°. A rat inoculated from the donkey on April 8 showed parasites on the 26th. The incubation period in the donkey was therefore ten days. Parasites were seen for the first time in its blood on April 18, and again on the 22nd.

Monkeys.—1. *Cercopithecus callitrichus.* Inoculated intraperitoneally on April 2 from a rat. Trypanosomes appeared in the blood on the 6th, and were constantly present for a week, when they became rare, occasionally being absent altogether. On the 7th the temperature rose to 105·5° F., and has since been irregular (with occasional rises to 104° to 105°, and over). No other symptoms have so far been observed, the appetite is preserved, and the animal is in good condition. 2. *Cercopithecus calitrichus.* Inoculated intraperitoneally on March 6 from a rat showing very few parasites. Trypanosomes did not appear in the blood before death, which took place from dysentery on the 10th. A monkey (*Macacus rhesus*), a rabbit, and one out of four rats inoculated with large doses of its heart blood became infected. Two guinea-pigs inoculated at the same time are still negative. Two mice inoculated with its blood died without becoming infected. 3. *Macacus rhesus.* Inoculated on March 10 intraperitoneally and subcutaneously with the heart blood of preceding monkey. Parasites were found in the blood on March 18, and were constantly present, although never very numerous, until April 6, when the animal died. On March 18 the temperature rose to 104·6°, with the appearance of parasites in the blood. During the last two days of life the temperature was subnormal (from 95° to 96°). Diminution of red cells and haemoglobin was observed. *Post-mortem*, purulent peritonitis was found due to a parasite found in the intestinal walls, and not yet identified. 4. *Macacus rhesus.* Inoculated on March 14 intraperitoneally with the heart blood of a rat. Trypanosomes appeared in the blood on the 18th, and were fairly numerous for four days ; on the 31st there were from thirteen to eighteen to a field. They became rarer again before death, which occurred on April 1. The temperature rose on the 18th, and on the 19th reached 105·8°, there being six parasites to a field at the time. The red cells decreased from 4,000,000 to 2,500,000 per cubic millimetre, and haemoglobin from fifty to twenty-seven per cent. 5. *Macacus rhesus.* Inoculated subcutaneously on April 22 with blood from a rabbit showing many trypanosomes. This monkey is not yet infected (April 27).

STRAINS DERIVED FROM THE BLOOD OF CASE 4[1, 2] AND FROM TWO CONGO NATIVES AT PRESENT UNDER OBSERVATION

Rats.—Incubation period from four to eleven days, average about seven days; duration, some of the animals have survived after eighty-seven days. In these animals trypanosomes have been scanty in the blood, with periods of some days to even weeks when none were to be found. This perhaps may be accounted for by the fact that the majority of the animals have been direct inoculations from the natives whereas the sleeping sickness and *Trypanosoma gambiense* strains have been passed through a series of animals.

Mice.—Parasites were always very rare and the duration was indefinite in direct inoculations. In mice inoculated from one of the guinea-pigs sent to us from the Congo Free State (see below), the incubation period was three days and parasites were more frequently present.

Guinea-Pigs.—Two guinea pigs inoculated by us directly from the natives, and two young guinea-pigs inoculated from monkeys have been negative for a period of one hundred and forty-six days. Two adult guinea pigs inoculated from Case 4[2] on the same day by the members of the Congo expedition, and sent home while still negative, have shown parasites for the first time in their blood on the seventy-ninth and one hundred and thirty-fourth days after inoculation. No symptoms have as yet been observed. They are still living one hundred and fifty-two days after inoculation.

Rabbits.—Incubation, direct inoculations nine and eleven days; sub-inoculation from rat ten days. Duration from twenty-one to eighty-seven days. Parasites have been fairly constantly present but only in small numbers. Loss of weight, slight rise of temperature, and anaemia have been the only symptoms observable.

Monkeys.—Macacus rhesus. Two have been inoculated intraperitoneally directly from the natives. Experiment 331. Inoculated on February 9. Parasites appeared in the blood on the fifth day. On the next day the temperature rose to 104·9° F., the following day it registered 105·8°; at the same time the parasites were observed to increase in numbers. Trypanosomes were constantly present at first. On March 5, there were from twenty-five to thirty to a field. About the middle of March the trypanosomes became scanty and were not found at all after March 17. Death occurred on March 25. Rises of temperature were observed in this monkey coinciding with temporary increase of numbers of parasites in the blood. Experiment 316. Inoculated on February 6. Parasites appeared on the 13th, and at the same time the temperature began to rise, and on the 19th it registered 104°, up to the end of March parasites were constantly present in its blood, since that date they have been scanty and sometimes absent. On March 27, marked oedema of the upper and lower eyelids, especially the left, was observed. This persisted for three days; the puffiness was confined entirely to the eyelids; no parasites were found in the oedema fluid. At

1. Received in a rat inoculated directly, and therefore in its first passage. 2. Dutton, Todd, and Christy, *loc. cit.*

monkey is ill it sits on its haunches with its head between its knees ; this position, which has been termed the typical sleeping sickness position, is not characteristic of trypanosomiasis or 'sleeping sickness' ; it is the position assumed by any sick monkey from whatever disease it be suffering. We have noted no nervous symptoms at all.

In stained specimens we have not observed any differences between the trypanosomes of Uganda and Congo sleeping sickness cases on the one hand and *Trypanosoma gambiense* (DUTTON) on the other. In the same species of animals and at corresponding stages of the infection the size and appearance of the former trypanosomes are precisely the same as those of the latter as described by DUTTON and TODD and since observed by us. In rats inoculated either directly or from monkeys inoculated directly they present the same stumpy and long forms, the former being more numerous in the early stages than in the later. After passage through some generations of rats the stumpy forms show the same tendency to disappear. In rabbits trypanosomes of all strains show the same preponderance of short stumpy forms as compared with rats, mice, and guinea-pig, while in guinea-pigs, so far as we have yet observed, there is the same greater tendency to length in most of the forms met with. In no animals infected with either the Uganda or Congo sleeping sickness trypanosome have we ever seen any form differing in measurements or appearance from *Trypanosoma gambiense.*

Pathological lesions.—In none of our animals have we found anything differing from the lesions described by DUTTON and TODD. Enlargement of the spleen is a feature in all the animals, more especially in rats and mice. In a guinea-pig, Experiment 133 (Gunjur strain), which died forty-two days after infection, rupture of the spleen was found, the rupture extending directly across the organ on its upper surface and near the posterior end for about one and a quarter inches. A profuse haemorrhage occurred and the animal bled to death. No history of an injury is known. Another guinea-pig inoculated with *Trypanosoma dimorphum* (DUTTON and TODD)[1] has died from rupture of the spleen. This case will be published in a forthcoming report on *Trypanosoma dimorphum.* The glands are very little, if at all, enlarged, and there are no haemorrhagic glands to be met with as in animals infected with *Trypanosoma dimorphum.* Petechial haemorrhages are rarely seen. In monkeys the mesenteric glands are enlarged, but this is without significance, as all our monkeys were infected with intestinal parasites. No macroscopical changes in the nervous system have been noted. In none of our animals, whether allowed to die or killed for special purposes, have we been able to find trypanosomes in the cerebro-spinal fluid, although the blood may have contained many trypanosomes, nor have any of the animals inoculated with this fluid ever become infected.

1 Laveran et Mesnil, *Comptes Rendus de l'Académie des Sciences*, tome cxxxviii, 1904, p. 732.

In the majority of animals which have died we are unable to say definitely that trypanosomiasis was the sole cause of death. Very frequently an intercurrent affection has occurred which, the animal's vitality having become impaired, has caused death. We would instance in rats broncho-pneumonia, caseous lung disease, and enteritis. In rats which have survived a few to many months it is exceedingly rare to find a single parasite,[1] nor has the negative blood appeared to be infective. Experiments are being conducted along these lines. In the majority of cases those rats which have been negative for months die without the presence of trypanosomes being again recognized. In some cases, however, rats which have been negative for months have at death once more shown parasites. As instances of this the following experiments are given.

Rat 95a[2] ('Q' strain) was inoculated on March 17, 1903, from horse Experiment 87; incubation period, eight days; parasites were found at irregular intervals and in small numbers. On September 13, 1903, a single parasite to a whole cover-slip preparation was seen, and the animal continued negative until its death on March 24, 1904, three hundred and sixty-five days after infection. At the necropsy a few parasites were found in the heart. Animals inoculated from it have not yet shown the infection.

Rat, Experiment 89[3] ('Lammin' strain). Inoculated February 26, 1903, from rat 26; incubation, eighteen days. Trypanosomes were present in its blood until April 21. From that time parasites were never seen except on the following dates, July 27, September 7, and November 1, 1903, until its death on March 15, 1904, three hundred and sixty-six days after infection. At the necropsy complete red hepatization of the whole of the left lung was found. The left pleural cavity contained over five cubic centimetres of slightly yellowish clear exudate containing one parasite to three fields. Animals inoculated with this exudate became infected, and from these sub-inoculations we have been able to infect a goat Experiment 518, and a monkey, Experiment 517.

We have observed that animals with extraordinary numbers of trypanosomes in their blood usually exhibit some lesion which tends to impair their vitality. The following experiment is a good example of this. A rat, 279a ('Gunjur' strain), inoculated on December 30, 1903, from a guinea-pig became positive on January 7, 1904. From that time until it was killed on March 22 the parasites were constantly present in increasing numbers in its blood. At the necropsy caseous lung disease was very far advanced, involving both lungs. Its blood on examination showed trypanosomes in countless uumbers. Two rats, two mice, three guinea-pigs, one rabbit, and a puppy were all inoculated intraperitoneally with heart-blood. Twelve hours later the mice and rats, the rabbit and two guinea-pigs were found to be positive, the rats and mice having as many as three to eleven to a field, the rabbit two hundred and forty to the cover-slip preparation, and the two guinea-pigs eight to the cover. The puppy did not show parasites in its blood until five days later.

Very little, if any, immunity is conferred by infection with *Trypanosoma gambiense*. The two following experiments are of interest in this connexion.

1. A piebald rat was inoculated directly from Mr. Q. by Dutton and Todd on November 3, 1902, Parasites were found in its blood on November 12, and again on February 27, 1903. After this the blood was always negative. On November 23, 1903, it was reinoculated intraperitoneally from a rat ('Gunjur' strain) showing very numerous trypanosomes. It became infected, and on December 9 its blood was swarming with trypanosomes. Death took place on December 26.

2. A monkey (*Macacus rhesus*) infected by Annett from H. K. (Dutton's original case) in 1902 recovered, its blood being non-infective to rats. On January 29, 1904, it was reinoculated (Experiment 311) from a rabbit ('Gunjur' strain), and became infected (see above).

As one would expect, there is no transmission of immunity to offspring. Several of the young rats which we have infected have been born from parents which either were infected at the time or had

1. This is also the case in other animals. See chimpanzee experiment.
2. Dutton and Todd, *loc. cit.*
3. *Ibid.*

recovered from an infection. And one guinea-pig (Experiment 216) which we infected with 'Gunjur' strain was born from a guinea-pig infected at the time (also with 'Gunjur' strain). There is a certain amount of natural immunity or resistance to the human trypanosome to be met with in individual rats and other animals. Every now and then we have come across animals which have exhibited this feature. For example, of two rats A and B of the same size, inoculated at the same time with equal amounts of virulent blood from an infected animal, A will become infected while B remains uninfected or shows a prolonged incubation period. Again, A receives a larger dose than B; it frequently happens that B will show the infection earlier than A. Guinea-pigs show considerable resistance to infection. As instances of this we would mention those inoculated from Rat 279A (see above). Baboons (*Cynocephalus sphinx*) have up to the present been absolutely refractory.

Conclusions

1. The trypanosomes found in (*a*) cerebro-spinal fluid of Uganda sleeping sickness cases, (*b*) cerebro-spinal fluid of Congo Free State sleeping sickness cases, (*c*) blood of Uganda trypanosome fever cases, and (*d*) blood of Congo Free State trypanosome fever cases, are all identical in animal reactions and morphology with *Trypanosoma gambiense*. The specific name *gambiense* (Dutton) must therefore for the future include the trypanosomes from the above-mentioned sources.

2. There seems to be no acquired immunity against infection.

3. There is no transmission of immunity to offspring.

4. An animal which seems to have recovered may months later show parasites once more, apparently as the result of lowered vitality.

TWO CASES OF TRYPANOSOMIASIS IN EUROPEANS

(Third Interim Report of the Expedition of the Liverpool School of Tropical Medicine to the Congo, 1903) *

BY

J. EVERETT DUTTON, M.B., VICT.

WALTER MYERS FELLOW, UNIVERSITY OF LIVERPOOL

JOHN L. TODD, B.A., M.D., C.M., McGILL

AND

CUTHBERT CHRISTY, M.B., EDIN.

THE following cases seem well worthy of record since they tend to indicate with what uniformity the accepted signs of the disease may be expected to occur in Europeans infected with *Trypanosoma gambiense*.

The completeness of the history of the onset of the disease in Cases I and II makes them particularly interesting. The history of Case I is compiled from notes and temperature charts taken by the patient's husband during her illness. Case II was seen very soon after the probable date of his infection.

CASE I

Mrs. G., *aet.* 35, a missionary. The patient's first stay in Congo was during the years 1895-99. During this period she had four small fevers, yielding to quinine—maximum temperature 103° F.; and a supposed attack of haemoglobinuric fever. One year without fever was then spent in England, and in June, 1900, patient, weighing then 147 lbs., again went to the Congo. Soon after her return she had three attacks of fever—maximum temperature, 104° F.—which were successfully treated by quinine; and from November, 1900, to July, 1901, she was in good health.

During the first days of August, 1901, the patient took a long canoe journey from Bolobo to her station at Bongandanga on the Lopori, and was severely bitten by 'river flies.' At the end of August, six days after the completion of the canoe trip, a severe fever commenced and lasted, without interruption, for twenty-five days. 'The highest temperature was 104° F., but the fever was very persistent and remained constant for two or three days at 103° F., then at 102° F., and so on. Quinine and phenacetin had no effect and the temperature was relieved only temporarily, but

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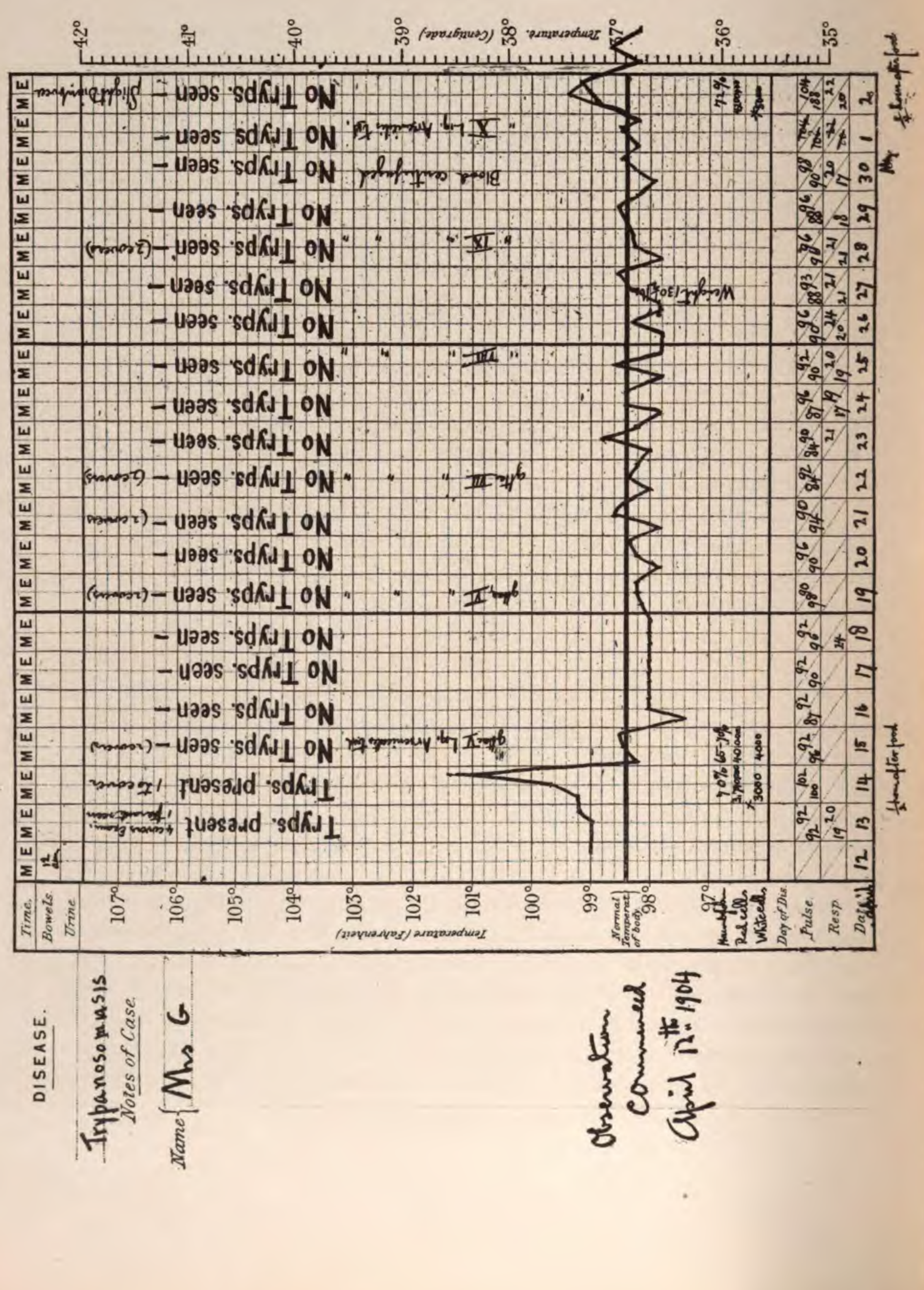
DISEASE.
Trypanosomiasis
Notes of Case
Name Mrs G
Observation commenced April 12th 1904
Tryps. present
No Tryps. seen
Blood centrifuged
Temperature (Fahrenheit)
Temperature. (Centigrade)
Normal Temperat. of body
Time.
Bowels.
Urine.
Day of Dis.
Pulse.
Resp.
Date

occasion eight times). There was no tenderness or pain, save headache, and no nausea or loathing of food. On the bad days an attempt at any exertion, even to read a letter, was certain to precipitate an attack of vomiting. About February 14 there was vomiting, once or twice, on alternate days. Took Liquor Arsenicalis, two to six drops three times a day.'

March 10. Stopped arsenic ; left her station for a change.

March 16. Headache and vomiting.

March 17. Severe vomiting, bile-stained vomitus.

March 18-20. Well.

March 22. Vomiting and headache, next day emesis more severe ; vomitus bile-stained.

March 24. Fairly well.

March 25. Severe headache, vomiting, fever (maximum 104·4° F.) The fever gradually lessened during the two following days, and from March 29 to April 12 patient was 'quite well.'

Quinine was given during this fever, but was, probably, never retained, since the ingestion of anything, liquid or solid, provoked emesis.

Physical examination.—April 15, 1904. General condition : Well-made woman ; height, 5 feet 6 inches ; weight, 126 lbs. ; rather anaemic appearance ; complains only of shortness of breath and of becoming easily fatigued.

Skin : No erythemata ; no distinct oedemas, although the skin of legs has a distinctly doughy feel ; on scratching skin capillaries contract.

Lymphatic glands : Slightly enlarged, palpable in axillae and neck.

Circulatory system : Slight venous pulsation in neck ; slight epigastric pulsation.

Heart : Apex beat normal in position ; cardiac dulness normal ; no thrill over heart or in neck ; sounds, a slight blowing systolic murmur limited to apex area, the aortic and pulmonary second sounds are rather loud ; no venous hum in neck.

Pulse : Frequent (96), tension medium, regular in time and force, artery normal.

Respiratory system : Nothing abnormal subjectively or objectively.

Digestive system : Appetite good ; bowels fairly regular, occasional slight constipation ; tongue clean.

Liver : Dulness normal, no tenderness on pressure.

Spleen : Not enlarged nor tender.

Nervous system : No headache or pain ; sensation to pain acute ; sensation to touch, heat, cold, and distance between pin points perfect ; there were distinct localized hyperaesthetic areas on different parts of the body, under right nipple, at apex of heart and on shins, slightest pressure causes acute pain. Superficial reflexes normal ; knee jerks just obtainable. Mental condition normal.

Eyes : Pupils react to accommodation and light ; both discs definitely congested (left more than right).

Throat : Tonsils not enlarged, no redness.

Generative system : Menstruation regular, flow normal in amount, generally slight temperature on first day.

Urine. April 29. Twenty-four hours specimen (preserved with thymol), volume 1,100 c.cm. ; acid, color normal ; cloudy precipitate, Sp. Gr. 1,020 (82° F.) ; no albumen or sugar ; urea, 1·85 grammes to 100 c.cm. urine.

April 30. Twenty-four hours specimen, volume 1,740 c.cms. ; color, normal ; acid, no albumin or blood ; urea, 1·55 grammes to 100 c.cms. urine.

Faeces. Careful examination showed no signs of intestinal parasites.

Liquor arsenicalis five drops three times a day was prescribed, and the patient was directed to increase the dose by one drop every third day. This treatment was followed until the 3rd of June (twenty drops t.i.d), when symptoms of saturation were evident, and this drug was discontinued for a time and iron and arsenic pills were substituted.

During 1895-96-97 quinine, grains V, was taken daily as a prophylactic against malaria. Its use was discontinued as it was thought to be the cause of 'neuralgia.' From January, 1903, to the present about three grains have been daily taken.

April 21. Slight epistaxis ; bleeding at the nose had occurred on three previous occasions, once in Switzerland, twice in Africa, always slight.

May 22. For past three days has had swollen wrists, first one then the other ; to-day one side of face is swollen, no redness or tenderness, or evidence of insect bite ; these swellings are fugitive, and last only for a day.

June 2. For last few days patient has complained of lack of appetite and loss of energy ; the swellings on face and hands persist ; a papular eruption has appeared on the chest.

The accompanying chart shews the type of temperature and the result of examinations for parasites during the first three weeks of our observations. On the 15th of April the parasites disappeared from the peripheral blood, and the temperature fell to normal. In spite of daily examinations of coverslips and periodic centrifugalization of blood, trypanosomes have not been again seen. The temperature (with one slight exception) has, during this period, always been normal.

Until signs of arsenical saturation commenced to show themselves the patient felt 'much better' ; she felt stronger and was less easily fatigued. There has been a slight increase in body weight, and in the value of erythrocyte and haemoglobin estimations.

May 12	May 27
Haemoglobin, 68 per cent.	74 per cent.
Red Cells, 4,510,000	4,450,000
White Cells, 4,650	4,000

We have never seen malarial parasites in this case.

It was through the kindness of Mr. M., a missionary stationed at Leopoldville, and the husband of the case 'Mrs. M.,' described by BRODEN and MANSON, that this case was brought to our notice. He felt convinced that he had, in October, 1903, seen an erythema on Mrs. G.'s forehead in every way similar to those which he had previously seen in each of the cases from the Congo described by MANSON.

We have been unfortunate in never seeing this case when erythemata were present.

CASE II

T., a steamer captain, *aet.* twenty-eight, serving his first term in Congo. Patient left Antwerp, November 26, 1903, and arrived at Leopoldville, December 30. He reached his station, a wood post for steamers, on the banks of the Congo, just below its junction with the Kasai, on December 31, and remained there until April 13, 1904, when he was invalided for 'fever' to Leopoldville.

Here he came under the care of Dr. Grenade, the State Physician, who found that quinine was without effect, and, suspecting the nature of the patient's fever, very kindly allowed us to study the case.

History.—Six years ago the patient spent eight to nine months in a cargo steamer plying between Sierra Leone and the Gold Coast; the steamer often remained in various ports for some little time. In spite of this he never had 'fever,' and in 1898 he returned to Europe. Since then, until leaving for the Congo, November, 1903, he had been employed on vessels plying between European ports. After frequent questioning, the patient admitted that during this period he had had very occasional 'fevers' preceded by a 'cold feeling,' and followed by sweating. No quinine was ever taken for these 'fevers.'

While on the East Coast of South America, during six months in 1894, he had yellow fever.

In 1901 he was in hospital for four or five weeks with rheumatism in legs and arms. With these exceptions, he has always had good health.

Complains of constipation since leaving Europe.

During his stay at the wood post he had five attacks of fever. The first commenced January 26, 1904, about four weeks after he reached the post, and lasted to February 3; during nine days he was ill, maximum temperature, 106·7° F.

The second attack occurred on February 17 and 18, two days' duration, fourteen days interval between it and first fever.

The third attack lasted two days, February 22 and 23, five days' interval between it and preceding fever.

The fourth fever also lasted two days, March 9 and 10, interval sixteen days.

During these last two fevers temperatures of 102·2° F. to 104° F. were recorded.

The fifth fever commenced March 17, and lasted eleven days, ending on March 27 ; temperature, 104° F. to 105·8° F.

The patient stated that the second, third, and fourth 'fevers' came on suddenly and without any premonitory signs ; for instance, while sitting at dinner he found himself in a high fever without any shivering fit or other prodrome. Before the first and fifth fevers, on the other hand, there was definite malaise, but no rigors.

From March 2 to April 10 he was alternately in bed and sitting in his chair ; on March 31 and April 1 and 5 his temperature was raised.

During these fevers and until his arrival in Leopoldville, April 13, he took about a gramme of quinine daily.

During the last fever he was greatly troubled by daily vomiting, which did not occur, however, unless something had been ingested.

The patient's friends say that he has lost flesh.

Physical examination.—April 18, 1904. Present condition : Man of average height and build ; distinctly sallow and anaemic appearance ; slight blepharitis (old-standing), complains of weakness ; says 'knees are weak ;' loses breath quickly.

Skin : Has a doughy feel, and is distinctly oedematous, this is most marked over ankles, where there is pitting ; there is no erythemata or other eruption ; dermatography not marked.

Lymphatic glands : Palpable, not enlarged.

Circulatory system : Heart, action rapid ; dulness, normal ; difference between first and second sounds, less marked than normal ; no bruits ; pulse, frequent, regular in time and volume ; artery, normal.

Respiratory system : Normal ; no complaint.

Digestive system : Bowels constipated, goes two to three days without movement ; tongue, flabby, not much furred, no tremor ; throat, normal ; appetite, fair.

Liver : Not enlarged (width in nipple line 6 cm.), a suspicion of tenderness.

Spleen : Just palpable ; slight tenderness.

Nervous system : Sensation to pain, touch, heat, cold, and distance apart of pin points, normal ; superficial reflexes, normal ; knee jerk, slightly increased.

Eyes : Left eye is seen by ophthalmoscopic examination to have a markedly congested disc, there is deep cupping ; choroidal vessels show very plainly.

Urine. April 29. Twenty-four hours specimen (preserved with thymol), volume, 660 c.cm., dark sherry color, acid ; urea, about 3 grammes to 100 c.cm. urine ; spectrum of urobilin, marked.

May 4. Twenty-four hours specimen ; volume, 1,075 c.cms., dark-brown sherry color ; acid, no albumen ; Sp.Gr. 1,016 (temperature, 79° F.) ; urea, 2·6 grammes to 100 c.cm. urine.

May 10. Twenty-four hours specimen ; volume, 700 c.cm. ; dark color ; Sp.Gr. 1,027 (temperature, 78·8° F.) ; acid, no deposit ; no blood or albumen ; urea, 2·6 grammes to 100 c.cms. urine.

April 30. Physical examination repeated, no change observed.

May 7. Patient drew attention to a redness on chest which he said was very obvious last night. On examination, red diffuse areas seen under nipple and on epigastrium, not itchy or painful ; one annular area about 5 cm. in diameter on right lower costal margin, had slightly swollen periphery with injected vessels, while the centre remained uncoloured. Five diffuse injected areas, varying in diameter from 2 to 4 cm., were seen on back.

May 8. Says that temperature rose to 104° F. at about twelve last night. To-day there is marked oval ring of faint, slightly raised erythema, measuring 1 by 5 cm. just above the right eye ; it is not tender or itchy, and the skin is distinctly thickened. The ring seen yesterday on right hypochondrium still persists, its capillaries are injected, and its general appearance is much the same as yesterday, its centre is yellowish ; there is no extravasation of blood (glass slide test) in either of these annular areas. On left hypochondrium were more or less blotchy areas of redness, mixed with very faint greenish or yellowish discolouration.

Patient has more colour and looks better than when he was first seen, he has more appetite and says that he feels stronger and does not become so easily fatigued as when he first came to Leopoldville.

May 9. The ring of erythema over right eye has almost entirely disappeared, the blotchy areas on body are vanishing. Although patient's temperature has been about 103° F. for the past two days, he says that he has felt no inconvenience from it. His appetite has been good, and he walked about as usual.

May 10. Patient leaves Leopoldville, invalided home. The remains of ringed erythema are seen on chest. Malarial parasites were not seen in this case.

The first attack of fever occurred within six weeks of this man's arrival in Congo. He spent approximately the first week in the Free State in Boma, and then went as directly as possible to his station. In twenty-seven days after reaching the wood-post his illness commenced. It does not seem at all probable that he acquired his infection so long ago as 1898 when he was on the Gold Coast, nor do we believe that he became infected either at Boma or while travelling by train to Leopoldville. We are inclined to think that he became infected with trypanosomes after his arrival at his post. It, therefore, seems probable that the 'incubation period,' between the time of infection with *Trypanosoma gambiense* and the appearance of the symptoms associated with human trypanosomiasis, may be so short as four weeks.

It is extremely interesting to note in this connexion that *Glossinae* were extremely numerous at this post, situated on the river bank, and were a most disagreeable and constant pest.

SUPPLEMENTARY NOTES ON THE TSETSE-FLIES
(GENUS GLOSSINA, WIEDEMANN)

BY
ERNEST E. AUSTEN*
ZOOLOGICAL DEPARTMENT, BRITISH MUSEUM; AUTHOR OF 'A MONOGRAPH OF THE TSETSE-FLIES,' ETC.

SINCE the publication of *A Monograph of the Tsetse-Flies* a little more than a year ago, our knowledge of these interesting insects, so important to the student of African trypanosomiasis, has been extended in various directions. It has, therefore, seemed advisable to embody these additions in a short paper, which it is hoped may not prove altogether unworthy of the attention of the members of this Section, and may serve to bring the author's Monograph so far as possible up to date.

In the work in question seven species of Tsetse-flies were recognized and described. But within the last few months an eighth species has been described under the name *Glossina decorsei*, by Dr. EMILE BRUMPT, from specimens recently obtained by Dr. DECORSE on the River Shari and the shores of Lake Chad.[1] An examination of some of Dr. DECORSE's specimens, however, kindly submitted by Dr. BRUMPT, shows that the supposed new species is in reality none other than *Glossina tachinoides* (WESTWOOD), which was described as long ago as the year 1850. In his Monograph, *Glossina tachinoides* was regarded by the present author as a variety of *Glossina palpalis* (ROBINEAU-DESVOIDY), the species that, since Colonel DAVID BRUCE's investigations in Uganda last year, has become widely known as the disseminator of *Trypanosoma gambiense*, now recognized as the cause of sleeping sickness. The study of further material, however, and especially of a long series of specimens obtained two months ago on the Benue River, Northern Nigeria, by Mr. W. F. GOWERS, and kindly presented by him to the British Museum, shows that *Glossina tachinoides* (WESTWOOD) is in reality a perfectly distinct species, nearly related to *Glossina pallidipes* (AUSTEN) but distinguishable at once, apart from its much smaller size, by the fact that the hind tarsi are either entirely dark, or, as in the female, are dark with the bases of the first three joints usually pale. The total number of species of Tsetse-flies now known

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therefore amounts to eight. In the paper already referred to Dr. BRUMPT considers that *Trpanosoma brucei*, the parasite of nagana or Tsetse-fly disease in domestic animals, is carried by at least five of these species,[2] and he further states that the investigations that he is making upon sleeping sleepness lead him to suppose that this malady may also be transmitted by several species of Tsetse-flies. The mere possibility that this supposition may ultimately prove to be true is perhaps sufficient warrant for thinking that no detail concerning the morphological characters, distribution, or bionomics of any of the species of *Glossina* is without importance for those interested in the sanitation of tropical Africa. In the present paper, therefore, the eight species will be considered in order, and any new facts regarded as worthy of notice recorded under each, the arrangement adopted in the Monograph being adhered to, and *Glossina tachinoides* inserted in its proper place. At the end of the paper a revised 'Synopsis of species' will be given, which it is hoped may prove useful for the determination of specimens.

Glossina palpalis (ROB.-DESV.)

The form of this species designated in the Monograph as 'Var. *tachinoides* (WESTW.)' must now be regarded as a variety which for the present may remain unnamed. It is characterized by the possession of pale femora, buff-yellow median stripe on the abdomen, and narrow pale hind margins to the abdominal segments. Specimens obtained by Drs. TODD and DUTTON on the Gambia during the Gambia expedition of the Liverpool School of Tropical Medicine belong to this form, which may eventually have to be raised to specific rank. A second variety also has the femora paler than usual; the palpi, except the tips, pale; and the abdomen somewhat reddish-brown, with the pale area on the second segment oblong instead of triangular. Owing to the colour of the abdomen this form presents a certain resemblance to *Glossina pallicera* (BIGOT). A specimen of variety No. 2 was obtained at Old Calabar on May 14, 1900, by Dr. ANNETT, of the Liverpool School of Tropical Medicine.

As many of the members of this Section are doubtless aware, our knowledge of the life-history of Tsetse-flies was originally due to Colonel BRUCE, who, during his investigations upon Tsetse-fly disease, or nagana, among domestic animals in Zululand in 1895, discovered that the species studied by him—either *Glossina morsitans* (WESTW.) or *Glossina pallidipes* (AUSTEN)—'does not lay eggs as do the majority of Diptera, but extrudes a yellow-coloured larva nearly as large as the abdomen of the mother.'[3] Specimens of the pupa of this species, kindly supplied by Colonel BRUCE, were described and figured on pp. 26-28 of the author's Monograph. Owing to the kindness of Colonel BRUCE, Captain E. D. W. GREIG, I.M.S., and Dr. NABARRO, all of the Sleeping Sickness Commission, who forwarded specimens from Entebbe, Uganda, it is now possible to describe the larva and pupa of *Glossina palpalis*.

Larva of Glossina palpalis

I have been enabled to examine a series of sixty-two larvae of this species, ranging in length from 2 mm. to 7 mm., but only one of these (the largest) can be regarded as adult. The colour of these specimens, which have been preserved in 5 per cent. formalin, varies from cream to buff yellow. In all the larvae the tumid lips on the last segment, as seen in the pupa, are plainly visible, and in a larva of $2\frac{1}{2}$ mm., which may perhaps be considered to be in the first stage (*i.e.*, before the first moult), the size of the lips is relatively, if not even actually, considerably greater than in a larva measuring $3\frac{1}{3}$ mm., which is perhaps in the second stage (*i.e.*, between the first and second moults). In the former stage, too, the lips are much wider apart than in the second, and the very young larva viewed from above may be said to be conical in shape, with a protuberance on each side of the truncate posterior extremity. In the second stage (larvae from 3 mm. to $3\frac{1}{3}$ mm. in length) the lips are nearer together, and separated by a much narrower interval than in larvae in the third stage (about $3\frac{1}{3}$ mm. in length and upwards), in which they have their final position, as seen in the pupa. In the first stage the lips, or anal protuberances, appear slightly brownish all over ; in the second stage they are blackish at their extremity and at the margin of the intervening notch ; in the third stage they are uniformly deep black, and the granules with which they are covered can now be easily discerned under an ordinary platyscopic lens. In the second and third stages the body of the larvae in front of the bifid anal extremity (twelfth segment) is seen to be composed of eleven clearly marked segments. In the larger larvae the tips of the mouth hooks can be seen slightly protruding from the cephalic end. The larva already referred to as being the only one that can be regarded as adult, measures 7 mm. in length, by 3 mm. in greatest width, and was obtained by Captain Greig, at Entebbe, Uganda, in April of the present year. In this larva, in the median ventral line, each of the segments from the fourth to the tenth shows on its anterior margin a narrow ridge, measuring about $\frac{2}{3}$ mm. in transverse width. The object of these ridges may be to assist the adult larva on extrusion to crawl away to some hiding place in which the pupal stage may be assumed.

All of the larvae here referred to were deposited by the parent flies in boxes, and, since all, with a single exception, are immature, it follows either that *Glossina palpalis* differs from the species described by Colonel Bruce, in that the larva feeds and grows outside the body of the mother after extrusion, or else that the parents of these larvae, probably owing to their being in captivity, gave birth to their offspring prematurely. The latter supposition would appear to be the more reasonable.

Pupa of Glossina palpalis

The pupae examined were all obtained at Entebbe, Uganda—those forwarded by Dr. Nabarro in September, 1903, while others were collected in April, 1904, by Captain Greig. Additional specimens were sent home last year by Colonel Bruce.

The pupa varies in length from $5\frac{1}{4}$ mm. to $6\frac{1}{2}$ mm., and in the greatest width from 3 mm. to $3\frac{1}{3}$ mm. In general appearance it is precisely similar to that of the Zululand species, figured on page 27 of the author's Monograph. The tumid lips on the last segment, however, are much closer together, the space between them being reduced by quite one-half, while the lips themselves are somewhat larger, and covered with sparser and therefore more conspicuous granules. The notch between the lips is somewhat deeper, and consequently approaches slightly closer to the preceding segment than in the species figured in the Monograph. Other points of difference are that the edge of each lip bears only two grooves or furrows instead of four, while the ridges connecting the lips at the base on the dorsal and ventral side, besides being lower owing to the greater depth of the notch, have a broad, shining black margin, instead of being almost dull.

Whatever be the case with regard to the larvae, it would seem probable that, did we but know them, all the species of Tsetse-flies might be distinguished in the pupal stage by the characters afforded by the last segment. At any rate, in a pupa forwarded from Entebbe by Captain GREIG with those already referred to, the lips are extremely low, much wider apart than in either of the forms previously mentioned, and separated by a wide and shallow notch instead of a deep and narrow one. The longitudinal grooves with which the connecting ridges are deeply scored at the base are very conspicuous, and the connecting ridges themselves are without a broad and shining black margin. This pupa, of which the dimensions are :—length, $7\frac{1}{3}$ mm., greatest width, 4 mm., should perhaps be assigned to *Glossina pallidipes* (AUSTEN), in which case the specimen figured in the Monograph would belong to *Glossina morsitans* (WESTW.).

DISTRIBUTION OF GLOSSINA PALPALIS

Since the publication of the author's Monograph, our knowledge of the distribution of this species has been considerably extended. As regards Uganda, BRUCE, NABARRO, and GREIG have published[4] a map showing the localities in which the species was obtained in the Uganda Province and Usoga. Here the fly is found on the forest-lined shore of Lake Victoria and the adjacent islands, passing down the Nile until Lake Albert is reached, all round which the species was met with last year by Mr. W. Y. WYNDHAM. In a letter to Dr. NABARRO, dated 'Wadelai, November 2, 1903,' Mr. WYNDHAM states that he has found the fly on all the shores of the Albert Nyanza, and also on the Congo side of the Nile, about eight miles to the south of Wadelai. He adds that from the results of his investigations 'there is little doubt that the fly is prevalent in this part of Africa, wherever the local conditions are favourable.' North of Lake Albert *Glossina palpalis* was encountered at Nimule, in the Nile Province of Uganda, by Dr. BRUMPT, who also found it to the west of the Nile in the Belgian enclave of Lado, and all down the Congo system, from the source of the Welle to the mouth of the Congo. Eastwards Dr. BRUMPT had previously met with the species on

the river Omo, which falls into the north of Lake Rudolf. In West Africa (Sierra Leone) Major FRED SMITH, D.S.O., R.A.M.C., writing to the author from Freetown on May 16 last, stated that in April of the present year he had found *Glossina palpalis* all the way from Freetown to Kakena in the north of the Sierra Leone Protectorate. It was the only species met with, though Major SMITH's native boys talked of a larger one (probably *Glossina fusca*), which, however, he did not encounter. In Northern Nigeria *Glossina palpalis* was found in March of the present year in the Kadima River Valley, thirteen miles south of Wushishi, by Dr. S. H. JONES, who kindly presented the three specimens collected by him to the British Museum. The National Collection has also received four specimens from Mr. W. F. GOWERS, collected by himself on the Forcados River, Southern Nigeria, on June 18, 1904. Lastly, it may be noted that a large series of specimens of this species were obtained by Drs. DUTTON, TODD, and CHRISTY near Leopoldville, on the islands in Stanley Pool, and at other localities on the Lower Congo from November, 1903, to May, 1904, during the expedition for the study of trypanosomiasis, recently dispatched to the Congo by the Liverpool School of Tropical Medicine.

Habits of Glossina palpalis.

Contrary to what has been found to be the case with regard to *Glossina morsitans* in South Africa, *Glossina palpalis* does not appear to be dependent for its existence upon big game, and in Uganda, at any rate, the members of the Sleeping Sickness Commission seem to have come to the conclusion that this species of Tsetse-fly subsists largely upon human blood. This is supported by the experience of Mr. W. Y. WYNDHAM on the Albert Nyanza. Writing to Dr. NABARRO from Wadelai on November 2, 1903, Mr. WYNDHAM says :—

'The fly cannot depend for its existence upon game, as in most of the places in which I found it there was none or next to none.'

On the subject of habits, Mr. WYNDHAM writes :—

'The fly seems a rapid feeder, to judge from some caught on the men. They do not appear early in the morning, but continue until evening has well set in, and I caught one which was decidedly lively after dark by candle-light.'

The following field notes concerning *Glossina palpalis* on the Congo have been kindly furnished by Dr. CUTHBERT CHRISTY :—

'We found *Glossina palpalis* to be extremely common on the banks of the Congo and its tributaries, even on the smallest streams, as far up as the mouth of the Kassi River. As to its presence beyond that I cannot as yet give you any information. We frequently observed that it was commoner and more bloodthirsty at bridges and fords, or in places where ferry canoes were kept, or where the women go down to draw water or wash, than on either side further up or down the stream. On the approach of either animal or man at a river crossing or in the densest forest, the victim is soon scented out by the Tsetse-fly if there be one in the vicinity, and then either silently or with a peevish buzz the fly makes straight for the most accessible spot, by preference the leg or foot in man, or in the ear in the pig, to which animal it seems especially partial.

'At Leopoldville we employed a brigade of boys, to whom we supplied nets and bottles. On wet or dull days their total catch was often not more than a dozen or so between them; but on other days from December to May they brought in regularly twenty or thirty each, the boys being paid according to the number each caught. The low-lying forest and scrub by the banks of the river was the best hunting ground. During the last week in January, by the kindness of the State authorities, who placed a steamer at our disposal, Dr. DUTTON and myself were enabled to make an exploratory tour of the islands in Stanley Pool. On the large forest-covered island of Bamou, where I spent two consecutive days hunting, *Glossina palpalis* were literally in myriads, and for the whole two days my hands, face, and neck were bitten unmercifully, seldom less than ten or a dozen flies attacking at the same time whenever I remained within one hundred yards of the abrupt edge of the forest. Surrounding this forest are areas of marsh many miles in extent, where patches of solid ground are few, and where not a Tsetse is to be found. In the dark, cool interior of the forest the Tsetse, although not nearly so numerous or so bloodthirsty as at the margin, were still a pest. On one occasion I counted thirty-eight probing the body of a large monitor (*Varanus niloticus*) that I had shot only a few minutes before. In the blood of this animal were numbers of *Drepanidia sp.* (*? nov.*), but no trypanosomes.

'On this island the question again rose:—"Has the buffalo any connexion with the Tsetse"? There were many small herds which passed backwards and forwards between the forest and the marsh, spending, however, most of their time in the marsh. One occurrence, namely, the fact that I came upon a herd of buffalo resting during the hottest part of the day, actually at the extreme edge of the forest, where, as I have said, the flies were, and always are in my experience, the most numerous and tormenting, tends to show that the skin of the buffalo, which is enormously thick, is too much for the Tsetse.

'Speaking from personal experience alone, the initial stab of *Glossina palpalis* is very painful, but no subsequent irritation follows. At the seat of the bite there soon appears a hard nodule, which may remain for many days, but is unaccompanied by any marked swelling or discoloration.

'I may add that at the end of April, I again visited Bamou Island, and was surprised on that occasion to find that *Glossina palpalis* was conspicuous by its almost total absence, hardly a fly being encountered, while the few that were seen appeared to have little inclination to bite. I have long suspected that this fly only sucks blood during certain months in the year.'

Glossina pallicera (BIGOT).

There is nothing to add to the account of this species given in the Monograph, and no further specimens have been received. The typical specimen, and a second one referred to on page 80 of the Monograph, have been kindly presented to the British Museum by Mr. G. H. VERRALL.

Glossina morsitans (WESTW.)

From a gravid female of this species taken near Yola, Northern Nigera, on October 10, 1903, by Mr. W. F. GOWERS, I have extracted an immature larva measuring 4 mm. in length by $2\frac{2}{3}$ mm. in greatest width. It is of the normal type, and the tumid lips on the last segment, which in this specimen are only slightly brownish in colour, are separated by a narrow notch but almost meet together at their margins, which appear to be marked by only two grooves.

Specimens of *Glossina morsitans* from one or two new localities have been received at the British Museum since the publication of the author's Monograph. These include seven males and five females from the Mwangazi River, on the borders of North-Eastern Rhodesia and Portuguese East Africa (latitude 14° 10′ S., longitude 32° 30′ E.) ; presented by Mr. ROBERT CODRINGTON, Adminstrator of North-Eastern Rhodesia. Besides these, Mr. W. F. GOWERS has forwarded a series of individuals of both sexes, taken by himself near Yola, Northern Nigeria, on October 10, 1903, and March 12, 1904. In a letter to Professor RAY LANKESTER dated 'Yola, October 21, 1903,' Mr. GOWERS writes as follows :—

'These specimens were caught about twenty-five miles north of Yola (the exact locality being 9° 36′ 40″ N. latitude, and 12° 41′ 20″ E. longitude), on the bank of the River Loko, a small tributary of the Benue, at the place where it is crossed by a well-used native path. There is here a belt of large trees with thick undergrowth, and, so far as the road is concerned, the fly appears to be confined to this belt, which is not more than one hundred yards across. I cannot yet say how far it extends into the bush to the east and west (the road runs nearly north and south). To the north, east, and south of this spot are many farms and villages. There are cornfields within two or three hundred yards, and a village not more than half a mile away. To the west is a stretch of uninhabited bush where game, including buffalo, is said to be plentiful, but there is no indication of game in the immediate vicinity, and it is not likely that it exists, at any rate in any quantity, in the neighbourhood of the road and cultivated land.

'The fly is found here in considerable numbers ; altogether about one hundred were secured in two hours by tying up a horse there as "bait."[5]. . . I have no absolute proof at present that the bite is fatal, but the local natives all agree that cattle, horses, and donkeys are killed by it, while it is harmless to sheep and dogs[6] ; the reason, however, given for the immunity of the sheep is that it is protected by its wool. I am going to test its effect on the latter two animals. Within a radius of three or four miles from the spot where the flies were caught no cattle are kept, the alleged reason being that the fly prevents it. Natives travelling with horses or cattle avoid this part of the road by making a detour to the east. Death is said to ensue from the bite in from one to three months, and from descriptions I gather that the symptoms are very much the same as in the case of fly-disease in South Africa. I fancy that Tsetse-fly will be found to be pretty well distributed throughout the southern portion of Northern Nigeria.'

In addition to the foregoing, a single female, presented by Colonel GRIFFITHS, Chief Veterinary Officer, Egyptian army, has been received from the Pongo River, between Wau and Dem Zibehr, in the Bahr-el-Gazal Province, Egyptian Soudan, where it was taken in 1903. Since this is another new locality for the species, it may be worth while to add that the place of origin is in Goro, west of the Rol country, near the intersection of the twenty-seventh parallel E. long. and eighth parallel N. lat., and that the fly is said to be very abundant there. Other specimens of *Glossina moristans*, collected by the donor in North-Western Rhodesia in 1899 and 1902, have been presented by Mr. VAL GIELGUD.

As regards the connexion between Tsetse-flies and big game in South Africa, it may be noted that Mr. ROBERT CODRINGTON, Administrator of North-Eastern Rhodesia in his Report on the Administration of North-Eastern Rhodesia for the year ending March 31, 1903 (Fort Jameson: Printed at the 'Administration Press,' North-Eastern Rhodesia), pp. 15-16, writes as follows :—

'The general increase of game of late years has been remarkable . . . The Tsetse-fly is now, for presumably the same reason, found in districts where it was before unknown.'

Similarly, on p. 23 of the same pamphlet, in a Report by the Civil Commissioner (CHARLES MCKINNON, Esq.) for the year ending March 31, 1903, on North Luangwa and Awemba districts, it is stated that :—

The Tsetse-fly is increasing to an alarming extent to the south of Mirongo, which I take to be due to the increase of game.

These observations support the statements on this subject in the writer's Monograph, pp. 14-15.

Glossina tachinoides (WESTW.)

As has already been stated, this species is nearly related to *Glossina pallidipes* (AUSTEN), which, except in size, it closely resembles, but is readily distinguishable by the colour of the hind tarsi. These are either entirely dark, as in the male, or have the first three joints somewhat lighter at the base. The front and middle tarsi are pale, with the exception of the last two joints, the tips of which are usually faintly brownish. The abdomen is marked with deep interrupted bands of dark brown, leaving the hind margins of the segments only narrowly pale. In the present paper there is no necessity to enter into a detailed description of this species, more especially since its diagnostic characters will be found in the appended 'Synopsis.' It may, however, be stated that it is the smallest of all the Tsetse-flies, the males not exceeding 6 to 6½ mm. in length, while the females measure 7½ to 8 mm., exclusive of the proboscis in each case.

In addition to the extensive series of specimens of this species, taken, as already stated, on the Benue River, Northern Nigeria, in the latter half of May and beginning of June of the present year by Mr. W. F. GOWERS, the British Museum has received, through the kindness of Professor MENSIL, of the Institut Pasteur, and Dr. BRUMPT, of the Laboratoire de Parasitologie, Paris, seven other individuals from the series collected on the river Shari, French Soudan, by Dr. DECORSE. Lastly, a single female obtained by himself thirteen miles south of Wushishi, in the Kadima River Valley, Northern Nigeria, at the beginning of last March (with three specimens of *Glossina palpalis*), has been presented by Dr. S. H. JONES.

As regards the occurrence of *Glossina tachinoides* on the Benue River, Mr. GOWERS has kindly contributed the following note :—

'This species of Tsetse-fly is found along the Benue River between Lau and Lokoja. With the exception of one or two small spots, no horses or cattle can be kept in this area. Above Lau, however,

the river banks are swarming with cattle, and there are large encampments of herdsmen in the dry season. After the rains have commenced the fly is present on the river in sufficient numbers to be an annoyance to travellers, and continually bites the canoe-men. In the dry season, however, which lasts from October to April, it is much less numerous.

'On the banks of the Benue River within the area in question almost the only species of game to be found is *Kobus kob*, which is very numerous indeed. West African buffalo, waterbuck, and reedbuck are met with in the swamps near the river; but in the Benue Valley there are, in the immediate vicinity of the river, more kob than specimens of all the other species of game put together.'

According to Dr. BRUMPT,[7] on the Shari River and on the shores of Lake Chad *Glossina decorsei* (that is, *Glossina tachinoides*) seems to be exclusively confined to the water's edge. The author in question further writes as follows :—

'The stab of *Glossina decorsei* is disagreeable, as is that of all the species of *Glossina*, but not very painful; it causes some time after the bite a rather acute itching. Both sexes feed on blood . . . The natives of the Shari dread the effects of the bite of the *Glossina* on their herds; like the inhabitants of many other countries, they have recognized the existence of a close connexion between the presence of nagana and the bite of this fly. Nagana is very widely spread on the Shari, where it was stated to occur by MOREL, and met with again by the Chevalier Expedition.'

Glossina pallidipes (AUSTEN) *and Glossina longipalpis* (WIED.)

There is nothing to add to the account of these species given in the Monograph. Additional specimens of the former, however, have been received from Colonel BRUCE, including six individuals collected by Dr. MOFFAT at Simba, East Africa Protectorate, in a carriage on the Uganda railway, and six examples from Busoga, Uganda. The latter is a new locality for *Glossina pallidipes*. In addition to the foregoing, a long series of *Glossina pallidipes* from Kibwezi, East Africa Protectorate, has been presented by Dr. Nabarro.

Glossina fusca (WALK.)

Since the publication of the Monograph the specimens of this species in the National Collection have been augmented by a large number of examples from Kibwezi, East Africa Protectorate, received from Dr. NABARRO. Besides these, five specimens, also from Kibwezi, collected in a railway carriage by Dr. MOFFAT, have been presented by Colonel BRUCE; while three specimens, obtained on the Congo by the Trypanosomiasis Expedition of the Liverpool School of Tropical Medicine, have been received from Dr. CHRISTY. Of the specimens last mentioned, which are all females, two were captured near Leopoldville, on December 26, 1903, and February 6, 1904, respectively, while the third was taken at Leisha on April 14 last. With reference to these flies Dr. CHRISTY has been good enough to furnish me with the following notes :—

'During our stay at Leopoldville three specimens of *Glossina fusca* were collected. One was brought in by a native alive, folded up in a leaf; another was captured among *Glossina palpalis* by our juvenile fly brigade, and the third was caught under the following circumstances :—On April 14, while sitting after dinner with two State officials, as late in the evening as 10 p.m., under the verandah of the Chef de Poste

of Leisha, a wood post some four days' steaming above Stanley Pool, I noticed one of the men, who as usual in addition to shoes and socks wore nothing on his legs more protective than a thin pair of trousers, holding between his finger and thumb a fly which I recognized as a Tsetse and immediately secured. It had been biting the man's ankle, and its abdomen was half full of blood. The man assured me that there was very little pain or irritation, but within ten minutes a large swelling arose obliterating the malleolus. In the morning this had somewhat subsided, but in its centre was a very distinct purple mark, as of a bruise, surrounded by a greenish-yellow area. During the next five day two more specimens of *Glossina fusca* were caught, I believe, by the same individual, at the same place and under precisely similar circumstances, but these I am sorry to say never reached me. Leisha wood post is on the bank of the river, surrounded by forest, and when I camped there for two days in April in the rainy season, *Glossina palpalis* and a large *Simulium* bit unmercifully from morning till night. At this post there were two advanced cases of sleeping sickness.

'A few days before the above occurrence, on my way up river on one of the small stern-wheelers, we found ourselves one afternoon tied up to the bank, while all the available hands on board were set to work to cut wood for our next day's steaming. At dinner that evening I wore, as a protection against mosquitoes, a pair of thin putties beneath my flannel trousers, and afterwards sat talking in the dark on deck. Towards 11 p.m. I felt a severe bite through the puttee, and, putting my hand to the spot, caught a Tsetse full of blood. By the light of the lamp in the saloon I recognized the fly as *Glossina fusca*, but unfortunately allowed the insect to escape while trying to put it into a tube. A quarter of an hour afterwards the swelling from this bite had extended nearly round my ankle. I experienced scarcely any pain or irritation, and in the morning the swelling had almost subsided, though the purple and yellow stain described above remained for days.

'On my return to Leopoldville Dr. DUTTON, before I had said anything about *Glossina fusca*, told me that while I had been away a certain official from higher up the Congo, who had taken some interest in biting flies, had, while visiting the laboratory, volunteered the information that the larger of the two species of 'mvakwa'—the native name for the Tsetse—bit at night, an assertion of the greatest interest in the light of my experiences up-river.'

It may, perhaps, be remembered that specimens of *Glossina fusca* found by Captain CRAWSHAY sitting on a path at Kaporo, at the north end of the Lake Nyasa, at sunset in February, 1895, did not bite (Monograph, p. 289). In view of Dr. CHRISTY's observations, their failure to do so cannot have been due to the fact that they were not met with in the heat of the day, as previously suggested by the writer (Monograph, p. 99).

Glossina longipennis (CORTI).

Of this species the only specimens received since the publication of the Monograph are a male and female collected in the East Africa Protectorate on the Uganda Railway in 1903, by Captain E. D. W. GREIG, I.M.S., and presented by Colonel BRUCE ; of these the male was obtained at Kibwezi station in thorny bush.

It was pointed out in the Monograph (p. 103) that *Glossina longipennis* 'is the Tsetse-fly of Somaliland and the adjacent regions, but that its range overlaps that of *Glossina fusca* (WALK.), somewhere in the vicinity of the Sabaki River.' Dr. BRUMPT states[8] that *Glossina longipennis* was the only species of Tsetse met with by him in Somaliland between July and October, 1901. According to the same author[9] this

species is responsible for the dissemination of a form of trypanosomiasis among camels and mules, which is probably identical with nagana, and, like the fly itself, is known to the Ogaden Somalis by the name 'aïno.'

The other species of *Glossina* considered by BRUMPT to be carriers of *Trypanosoma brucei* in the case of domestic animals were referred to at the commencement of this paper, and it may be pointed out in conclusion that this author believes[10] that, in addition to trypanosomiasis in its various forms, Tsetse-flies must play an important part in the dissemination of other diseases due to haematozoa. BRUMPT states that in certain districts on the Upper Congo a filariasis due to *Filaria volvulus* is very widely spread; the disease occurs only among the canoe paddlers—that is, among those who are most exposed to the bites of Tsetse-flies. 'The only cases hitherto known,' writes BRUMPT, 'have been observed in the regions (such as Nigeria and Dahomey) in which Tsetse-flies abound. The lymphatic tumours caused by *Filaria volvulus* are met with, especially in the places towards which the lymphatics of the exposed regions converge.'

REVISED SYNOPSIS OF THE SPECIES OF GLOSSINA

1. Hind tarsi entirely dark, or at least all the joints more or less dark (in the ♀ of *Glossina tachinoides* the basal half of the first joint and the extreme bases of the following joints are usually pale) 2
   Hind tarsi not entirely dark; last two joints alone dark, remainder pale 4
2. Ground colour of abdomen ochraceous-buff, with interrupted dark-brown deep transverse bands, and sharply-defined pale hind borders to the segments; a very conspicuous square or oblong pale area in the centre of the second segment; small species, not exceeding 8 mm. in length (exclusive of proboscis), the males considerably smaller ... *tachinoides*, WESTW.
   Abdomen not so marked, very dark or for the most part uniformly brown, hind borders of segments if lighter extremely narrow and cinereous; pale area in centre of second segment usually triangular, with the apex directed backwards and continued into a cinereous median stripe; larger species 3
3. Third joint of antennae dusky brown to cinereous black ... ... *palpalis*, ROB.-DESV.
   Third joint of antennae pale (orange-buff) ... ... *pallicera*, BIGOT.
4. Large species: length at least 11 mm. ($5\frac{1}{4}$ lin.), wing expanse (measured from tip to tip, when wings are set at right angles to body) at least 25 mm. ($11\frac{3}{4}$ lin.) 7
   Smaller species: Length rarely reaching 11 mm. ($5\frac{1}{4}$ lin.), often considerably less; wing expanse not exceeding 25 mm ($11\frac{3}{4}$ lin.). 5
5. Last two joints of front and middle tarsi with sharply defined dark-brown or black tips ... 6
   Last two joints of front and middle tarsi without sharply defined dark-brown or black tips—front and middle tarsi entirely yellow, or last two joints of former faintly tipped with pale brown ... ... *pallidipes*, AUSTEN.
6. Generally distinctly larger; head wider; front darker and narrower in both sexes, sides parallel in ♂; abdominal bands deeper, leaving hind margins of segments only narrowly pale; hypopygium in ♂ smaller, darker, and more hairy; tip of ♂ abdomen more thickly clothed laterally with short black hair, bristles on sixth segment finer and less prominent ... ... *longipalpis*, WIED.

Usually smaller ; head narrower ; front paler and wider ; eyes in ♂ as well as in ♀ distinctly converging towards vertex ; abdominal bands less deep, pale hind margins of segments therefore deeper ; hypopygium in ♂ larger, paler, somewhat more oval in outline, and clothed with fewer fine hairs ; tip of ♂ abdomen less hairy laterally ; bristles on sixth segment in ♂ stouter and more conspicuous... ... ... *morsitans*, WESTW.

7. Dorsum of thorax with four sharply defined small dark-brown oval spots, arranged in a parallelogram, two in front of and two behind transverse suture ; bulb at base of proboscis brown at the tip ... ... *longipennis*, CORTI.

Dorsum of thorax without such spots, though with more or less distinct longitudinal stripes ; bulb at base of proboscis not brown at the tip* ... ... *fusca*, WALK.

NOTES AND REFERENCES

1. Sur une Nouvelle Espèce de Mouche Tsétsé, la *Glossina decorsei*, n. sp., provenant de l'Afrique Centrale. Par M. E. Brumpt (*Comptes Rendus des Séances de la Société de Biologie*, Séance du 16 Avril, 1904), T. lvi, p. 628-630.

2. *Glossina Longipennis* in Somaliland ; *Glossina morsitans* and *Glossina pallidipes* in Zululand and elsewhere ; *Glossina palpalis* in the basin of the Congo ; and *Glossina tachinoides*, Westw. (*Glossina decorsei*, Brumpt) on Lake Chad and the Shari. Mr. Gowers also found that the latter species carries the disease on the Benue River.

3. *Preliminary Report on the Tsetse-fly Disease or Nagana in Zululand.* By Surgeon-Major David Bruce, A.M.S. Ubombo, Zululand, December, 1895 (Bennett and Davis, Printers, Field Street, Durban), p. 2.

4. *Reports of the Sleeping Sickness Commission*, No. iv, November, 1903.

5. Mr. Gowers has recently informed me that this horse died in three weeks, undoubtedly from Tsetse-fly disease. —E. E. A.

6. In South Africa dogs, at any rate, succumb to Tsetse-fly disease.—E. E. A.

7. *Loc. cit.*, p. 629.

8. *Comptes Rendus des Séances de la Société de Biologie* (Séance du 23 Avril, 1904) T. lvi, p. 673.

9. *Ibid* ; see also *op. cit.*, T. lv, p. 1497.

10. *Op. cit.*, T. lv, p. 1497.

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* N.B.—The ordinary dark-brown patch on each side of the bulb on its upper margin, which is often especially well marked in West African specimens, must not be mistaken for a brown tip.

A NEW HAEMOGREGARINE IN AN AFRICAN TOAD

TWO CASES OF INTESTINAL MYIASIS

NOTE ON THE PATHOLOGY OF TROPICAL 'SWELLINGS'

NON-FLAGELLATE TYPHOID BACILLI

A NEW HAEMOGREGARINE IN AN AFRICAN TOAD

By J. W. W. STEPHENS, M.D., Cantab., D.P.H.
WALTER MYERS LECTURER IN TROPICAL MEDICINE, UNIVERSITY OF LIVERPOOL

OUT of a batch of toads received from Sierra Leone, in October, 1903, five were examined by me. Four out of the five were infected with haemogregarines.

In one toad they were with difficulty found, in the others the haemogregarines were fairly plentiful. At a first examination it struck me that the forms present differed from any of those described as occurring in reptiles or amphibians. I consequently sent a slide to Professor E. A. Minchin, who, from a preliminary examination, also agreed that the parasites probably belonged to the genus haemogregarina in the sense used by him in his monograph on the sporozoa in Lankester's Zoology, and that they probably belonged to a new species. I consequently determined to describe the parasites, especially as I found it difficult to keep the toads alive.

It may be well first to describe the classification used by Minchin.

Order Haemosporidia (Danilewsky).

Sub-Order I. Haemosporea.

Genus I. *Lankesterella* (Labbé, 1899) for *Drepanidium* (Lankester). The haemogregarine is not more than three-quarters the length of the blood corpuscle it inhabits.

Type—*L. ranarum* or more correctly *L. minima* (Chaussat).

Genus II. *Karyolysus* (Labbé, 1894), The haemogregarine does not exceed the corpuscle in length.

Type—*K. lacertarum* (Danil.) from lizards.

Genus III. *Haemogregarina* (Danilewsky, 1897) (syn. *Danilewskya*, Labbé, 1895). The body of the parasite when adult exceeds the corpuscle in length, and is bent on itself within it, in a characteristic manner like the letter U.

H. lacazei in lizards is the commonest.

H. magna is the only haemogregarine in the strict sense so far described in amphibians, and it is possible that this is the macrogamete of *Lankesterella*, *sp.*

The parasite, as seen in stained specimens, presented itself under two forms. Whether we are dealing with two haemogregarines or developmental forms of the same species I am unable to declare with certainty, but incline to the former view. as the two forms were practically constant in shape and staining reactions, and I could not observe intermediate stages.

1. The first form consists of a parasite only slightly longer than the red cell, its pointed end being bent into a slight crook (Fig. 1).[1] In this form there is not observed the great enlargement and decolouration of the red cell seen in the case of the second form. With ROMANOWSKY the nucleus stains typically, reddish rose colour, and the protoplasm shows a number of coarse reddish granules mainly collected at the pointed end. In its staining the nucleus differs from that of the other form, which very rarely is stained red but only blue. The nucleus of the cell is displaced, but not nearly to the same extent as in the second form. Younger forms than this I have not observed.

2. The second form consists of a typically folded vermicule (Fig. 2). They vary somewhat in size, and in the larger forms several peculiarities of structure are noticeable.

The parasite itself has a well-developed nucleus situated in the thicker half of the parasite in the stained specimen. The nucleus commonly has a rhomboidal or elongated triangular shape (Fig. 5). It stains blue with ROMANOWSKY and very rarely red. Not uncommonly at the tip of the 'tail' end of the parasite there is a blue stained spot (Figs. 2 and 3). The large **U**-shaped forms are surrounded by a well-defined cyst which separates the parasites from the cyst wall. This cyst frequently shows some peculiarities of structure ; at the end corresponding to the bend of the haemogregarine there is an area staining a deep dull red (Fig. 3). This area is concave on the side facing the parasite, so that it appears to fit over the parasite as a cap. It is not, however, actually attached to the parasite, for sometimes a clear line can be seen separating this reddish area from the parasite (Figs. 4 and 5). The larger forms of the **U**-shaped parasite invariably show this stained (capsular) area, but it is not developed in the smaller forms, when also the cyst is not clearly defined. A certain amount of staining reaction (red) also is seen in other portions of the cyst. It is, however, in these encysted forms of the parasite, when found entirely free from red cells, that the cyst contents surrounding the parasite show a more diffuse staining. In these the conical area, at one end, is often lost and instead the cyst shows a general mottling with rather coarse granules (Fig. 6).

Forms diverging somewhat from these typical forms are occasionally seen. Thus—

(i) We have forms showing as many as three adjacent nuclei, with indications of a corresponding division of the protoplasm (Fig. 7).

1. The figures illustrating this paper have unfortunately been lost.

(ii) Rather large spherical forms with a single nucleus, and no lines of division visible, and no development of a conical red area within the cyst wall. With regard to the cyst wall, this is of a well-marked double outline, and in the free forms shows perforations (Fig. 8) or intervals at certain points.

(iii) Free encysted forms in which the outline of the parasite is almost lost, and the contents consist of a finely granular reddish mass in which denser masses of chromatin occur (Figs. 9 and 11).

In some of these toads the leucocytes contained masses of pigment consisting of numerous spherical granules.

In fresh specimens of blood no motile vermicules were encountered. In some respects this parasite resembles *H. stepanowi*, but this has only been recorded in reptiles.

Since this was written NICOLLE and subsequently BILLET have described a haemogregarine in toads (*Bufo mauritanicus*) from Algeria.

Though the description of the cyst and its polar masses of stain differ in some respect from what I have seen in these specimens, yet there are so many similarities in the parasite itself that it is probable that the species are identical or closely allied. It will be therefore inadvisable at present to propose a name for this West Coast haemogregarine.

TWO CASES OF INTESTINAL MYIASIS

By J. W. W. STEPHENS, M.D., Cantab., D.P.H.
WALTER MYERS LECTURER IN TROPICAL MEDICINE, UNIVERSITY OF LIVERPOOL

THE larvae to be described in this paper were procured from two young children in Liverpool, in October, 1903. In both cases the larvae were stated to have been passed per rectum.

In the first case the larvae of two different species were present, viz., those of *Musca corvina* and *Homalomyia canicularis*. In the latter case the larvae of *Homalomyia canicularis* only. The latter larvae were brought alive to the laboratory, and an attempt was made to hatch them out by keeping them on a decomposing mass of faeces, flesh, etc., but as the larvae crawled out of this material on to the sides of the jar, they were subsequently, at the suggestion of Mr. Theobald, of the British Museum (Natural History), put in damp moss. They buried themselves in this. The jar containing them was covered with mosquito netting, and put in the hot incubator. About eight larvae were put in, and in about a month's time two flies had hatched out within a few days of one another. The flies were kindly identified by Mr. Theobald as *H. canicularis*, and the identification of the larva was thus established; previously, Mr. Theobald had only been able to say that the larva belonged to the genus *Homalomyia*. As in the literature of this subject consulted by me the specific identification is rare, and as very few descriptions of the larvae are given, I have thought it well to describe the main distinctive features of the larvae.

Larvae of M. Corvina.

The larva varies in size from four to ten millimetres, is whitish in colour, thick, and bulbous at one extremity, tapering at the other end, and here a dark speck is seen with the naked eye, which under the microscope proves to be a single curved dark hook (Fig. 3).[1] The larva consists of eleven (?) segments, the head being retracted in the formalin specimens. On the head segment three antennae-like processes exist, apparently consisting of three segments, but as these were retracted it was difficult to be certain about this. The most distinctive features are the lateral respiratory processes on the anterior end. They consist of eight processes on a somewhat thick column (Fig. 2). The ends of the processes are slightly bulbous, and appear to be really discs. The number eight distinguishes these larvae from those of *M. domestica*, which, according to Theobald, has seven only.

1. The figures illustrating this paper have unfortunately been lost.

The larva possesses slight inconspicuous spines at the joints of the segments. On the anterior segment it is marked with pits, while elsewhere there is a reticular pattern.

On the terminal bulbous segment of the larvae are situated two beautifully adorned structures, the openings of the respiratory syphons (Fig. 1). These are like two discs without any perceptible stalk, with the margin turned slightly downwards. The inner portion is marked by a beautiful convoluted (chitinous) yellow marking (peritreme), which is interrupted at one point, and in the larvae examined the interruptions in each convolution are at corresponding points, so that they face each other. In the larger larvae this convoluted arrangement could not be clearly traced, and a central dark area of the discs noticeable in the large larvae was absent in the small. In the larger larvae the syphon seemed to consist of a disc, on which was situated a little mole hill (area of convoluted marking), and in this was a small central crater (Fig. 4).

On the anterior end of the larvae could be made out a pattern of more or less elongated spaces passing round the larva.

The larvae of *Musca corvina* are then characterized by

1. The single hook.
2. The polygonal areas (seen anteriorly).
3. The lateral respiratory appendages with their eight processes.
4. The posterior respiratory syphons (2) with their convoluted tracings.

Larva of Homalomyia canicularis.

They differ much in appearance from the former. They are of a brownish colour, about 6 mm. in length, pointed at the anterior extremity and rounded posteriorly (Fig. 1). They have a dorsal convex surface and a flattish ventral surface. To the naked eye they appear hairy. On examination with a low power of the microscope the larva is seen to possess thorny appendages on each segment. There are two sets of main appendages—a dorso-lateral and a ventro-lateral pair on each segment. The dorsal pair of hairs are somewhat coarse and longer than the ventral pair. Further, on the true dorsal surface between the dorso-lateral hairs exist a short pair of central hairs on each segment (Fig. 2). The hairs are attached in the hind segments to the posterior margins of the segments, but, as we proceed anteriorly, they shift their position gradually, so that in the anterior segments they are attached to the anterior margin. The hairs, on examination, are seen to be markedly spinous (Fig. 2), coarse at the base, decreasing in size towards the tip, and there are about twenty main thorns on each process. They are arranged in two rows—anterior and posterior.

The lateral margin of the segments, from which the hairs proceed, are also coarsely spinous. Besides these principal segmental appendages, there are the smaller spinous dorsal hairs already mentioned, but we also have between these and the lateral

NOTE ON THE PATHOLOGY OF TROPICAL 'SWELLINGS'

By J. W. W. STEPHENS, M.D., Cantab., D.P.H.
WALTER MYERS LECTURER IN TROPICAL MEDICINE, UNIVERSITY OF LIVERPOOL

THE patient, the subject of this note, was seen by me in the autumn of 1903, in the tropical department of the Johnston Laboratory, Liverpool University. He was a patient sent by Dr. Fletcher, Degama, Southern Nigeria. In his letter, dated, June 1903, Dr. Fletcher says, 'He has for some months been suffering from cellulitis, or at least peculiar swellings in his hands, arms, feet, and face, *i.e.*, either one or the other; sometimes only one of these is affected at the same time, sometimes two. I know Calabar swellings, but it is not like that; I made several attempts to find a filaria but failed.' The patient informed me that he had first noticed the swellings in March, 1903. The swellings he described as thickenings, hard in character and not pitting. They occurred mainly on the hands, also once below the eyelid. They had occurred on the leg, and then covered an extensive area above and below the left knee, and according to his statement only one swelling occurred at one time at one place.

When the patient was seen by me, in answer to my question, he stated that at that time he had no swellings on any part of his body, but when his attention was called to a slight swelling on the ulnar side of his wrist (left) about as broad as a crown piece, he stated that the swelling was of the same nature as the others he had suffered from. The swelling was not well marked, it was but slightly raised, somewhat elastic in consistency, and certainly was not hard, nor was there any redness or oedema. A careful inspection of his body revealed nothing further. His temperature was not raised.

A blood examination was made for parasites with ordinary and thick films. The result was negative. In examining for parasites, however, it was observed that there was a distinct eosinophilia. Careful counts were then made of a number of slides, with the following results :—

Leucocytic count (600 counted)—

Large mononuclear	1
Small mononuclear	23
Polynuclear	26
Eosinophil	50

Such a marked relative increase in eosinophil cells suggested a worm infection of one kind or another. A number of blood films, thick and thin, were sent to the laboratory by the patient, the blood being taken at night and day, but no filarial embroyos were found in the blood. The faeces were also examined for ova of worms, but the result was negative. The blood from the swelling itself was also examined with negative result.

I have thought it important to place on record this condition of the blood in this case so that the blood of cases of 'Calabar swelling' may be likewise examined. The nature of this case, however, is quite obscure, and I have called it 'tropical swellings' for lack of a better name, and because Dr. FLETCHER did not consider it to be one of 'Calabar swelling.'

I need not here discuss the views held as to the nature of these latter, but have contented myself with recording this remarkable condition of the blood in a possibly identical or allied condition.

NOTE.—WURTZ and CLERC,[1] in a case of *Filaria loa*, have also found a marked eosinophilia. At no time were embryos found in the blood. Their count of five hundred leucocytes was as follows :—

Large mononuclear - - - -	5 per cent.
Small mononuclear - - - -	13 per cent.
Polynuclear - - - - -	29 per cent.
Eosinophil - - - - -	53 per cent.

It is interesting to note also that there was a marked leucocytosis.

$$\frac{WC}{RC} = \frac{19{,}600}{4{,}100{,}000}, \textit{ i.e., } \frac{WC}{RC} = \frac{1}{209} \left(\frac{WC}{RC} = \frac{1}{500} \text{ normal value}\right).$$

1. *Compt. Rend. Soc. de Biol.*, p. 1,704, 1904.

NON-FLAGELLATE TYPHOID BACILLI

By J. W. W. STEPHENS, M.D., Cantab., D.P.H.
WALTER MYERS LECTURER IN TROPICAL MEDICINE, UNIVERSITY OF LIVERPOOL

In an article in the *Thompson Yates Laboratories Report*, 1903, I described a method of staining flagella with silver. Shortly afterwards I proceeded to demonstrate the method to a class of students, and to my great surprise and annoyance I failed to show any flagella at all in the culture of typhoid bacilli used. I made many attempts, but all were negative, and I naturally concluded that there must have been some factor at work in my earlier successes which I was now unable to repeat. I feared, too, that I had given publicity to a method which was uncertain, since I myself was now unable to get positive results. I then set about discovering what procedure in my previous method had escaped me, and I made a large series of tedious experiments extending over many months in attempting to determine exactly in what respect my method was now wrong. But all to no purpose, I failed to stain a single flagellum. The suspicion then gradually arose in my mind that the culture might be to blame. I consequently stained three different laboratory cultures of typhoid, but again all were negative with regard to flagella. I then felt that the culture was not to blame but the method, and I once more applied myself to repeating my original method and to making all kinds of modifications, but again with negative results. It then occurred to me to examine my cultures (fresh) in the hanging drop, and to my surprise I found that none of them exhibited the typical motility of typhoid bacilli, but they only showed 'trembling' movements. I was not aware at the time that typhoid bacilli could become practically non-motile, but on consulting the literature I found that this was recognized. I could find no statement, however, that this 'non-motility' was associated with loss of flagella, and it appeared to me now possible that my numerous failures were due not to a faulty method but to the fact that the bacilli had really no flagella. I consequently procured a freshly isolated typhoid bacillus which showed active motility, and proceeded to stain it by my method. I had no difficulty in demonstrating flagella, and repeatedly did so without any failures. So I felt justified in concluding that my previous cultures had no flagella, and that was the reason of my repeated failures. These cultures were from old laboratory strains some years old, and were those used for serum diagnosis of typhoid fever, and I was informed that they reacted normally

in that respect. Experimenting now with one of these cultures, non-motile and non-flagellate, I attempted to restore its motility and flagella, and I grew them for a long time on glycerine and dextrose solutions. But so far the motility has not been restored. I then passed this culture through a guinea-pig, once, and isolated the bacillus from the hearts blood. The bacillus examined was now *motile*, and had all the cultural characters of typhoid. On staining for flagella the result was *positive*.

These observations seem to me to prove not only that we have non-flagellate typhoid bacilli, but that the flagella can be restored by 'passage' through animals. I think this is of importance in many respects.

1. It shows that non-motility and the non-flagellate condition may not be characteristic of the bacillus, but may be an acquired character due to laboratory conditions of growth. How far other bacilli can lose their flagella remains to be seen. For instance, a very old strain of *B. pyocyaneus* examined by me was found to be actively motile, and, of course, to possess flagella.

2. It will be necessary in certain cases before we can state that a bacillus is non-flagellate to examine it as soon as isolated from the body, or to pass it through an animal. Thus in the case of *B. dysenteriae* and other bacilli and cocci there is a certain conflict of evidence as to the presence or absence of flagella, which may be due to the age of the culture.

3. If this bacillus 'reacted normally' in the agglutination test of typhoid serum then the presence of flagella cannot be an essential factor in agglutination.

4. Biologically, the experiment is of interest as showing how profoundly environment can modify structure.

I may refer, in conclusion, again to the method used by me—it is a simplification and modification of VAN ERMENGEM's silver method.

1. The mordant is osmic acid and tannin (I find also that $FeSO_4$ and tannin will give positive results ; but I have, so far, only made one observation). This is allowed to act for one-half to one hour or more.

2. Silver solution, 0·1 to 0·3 per cent.

3. Ammonium tannate solution :—

   2/10 c.c. 20 per cent. tannin - - - - }
   1 c.c. (H_2O and NH_3 fortiss : equal parts) - - } freshly made.

This solution is a yellowish red colour. A little silver solution is placed on the slide. Then some tannate. It is rocked to and fro for a short time and then washed off with water. The process is repeated three or four times until the film appears deep brown or black. This formula is somewhat different from my previous ones, but the exact proportions do not seem very important, except that it is easier to obtain absolutely clean specimens with the strong ammonia solutions. The flagella are beautifully stained. The emulsion used should be just faintly milky, and I find it best to flood the slide with the solution and then allow it to drain away.

CLADORCHIS WATSONI (CONYNGHAM)

CLADORCHIS WATSONI (CONYNGHAM)

A HUMAN PARASITE FROM AFRICA

By A. E. SHIPLEY, M.A., F.R.S.

FELLOW AND TUTOR OF CHRIST'S COLLEGE, CAMBRIDGE, AND LECTURER ON THE MORPHOLOGY OF THE INVERTEBRATA IN THE UNIVERSITY

I. HISTORY AND ORIGIN OF THE PARASITE

THE external features of this Trematode have been described by Mr. H. F. Conyngham.[1] The history of the parasite is given in the following account kindly furnished me by Dr. J. W. W. Stephens, to whom Dr. Watson communicated it :—

> 'The patient was a Pagan who had come from Adamawa, German West Africa—one of a gang of freed slaves brought to the Resident of Zola, Northern Nigeria, nearly all of whom were in a terrible condition due to starvation.
>
> He made a certain amount of progress at first, but did not improve as the others, and had constant diarrhoea. The stools were watery and of a bilious colour, no blood or mucous in the same. He was taken into the hospital but died the same night, and on inspecting the stools passed during the night numerous reddish-yellow, translucent, gelatinous, oval bodies were found.
>
> *Post-Mortem.*—The lungs and heart were normal. Liver normal. The spleen small, hard, and black. The stomach contained some food, and on opening the small intestine the duodenum and upper part of the jejunum were found full of the oval bodies, none of them adherent, although they were alive. The mucous membrane was reddish, but no haemorrhages or petechiae were apparent. The rest of the bowel was normal, a few of the oval bodies being found loose in the large intestine. The kidneys were normal. The oval bodies have shrunken considerably, and are only about a third of the normal size.
>
> These Pagans appear to be extremely fond of raw meat, and eat fowls raw.'

1. *B. M. J.*, No. 2,281, Sept. 17, 1904, p. 663.

I am greatly indebted to Dr. STEPHENS for supplying me with the material upon which this paper is based.

I have discussed the systematic position of this parasite at the end of this article. Here I will only mention that it belongs to the family *PARAMPHISTOMIDAE* (FISCHOEDER) and to the sub-family *CLADORCHINAE*. Allied to it and belonging to the same sub-family is the genus *Gastrodiscus*, which is represented amongst the human parasites by *Gastrodiscus hominis* (LEW and McCONN).[1] This latter seems to have been observed but twice in the human body—once in a native of Assam and once in an Indian. It occurred in great numbers in the colon and coecum. Its normal host is probably the genus *Equus*, both in Africa and in India.

It seems reasonable to surmise that in the case of *Cladorchis watsoni* also man is but an occasional host, and that the normal host is some herbivorous animal. We know nothing of the life history of either form, but from our knowledge of trematodes in general it seems improbable that the habit of eating raw meat has anything to do with the presence of the parasite in the human intestine. *Gastrodiscus hominis* and *Cladorchis watsoni* are the only members of the family *PARAMPHISTOMIDAE* hitherto found in man. With the exception of *Schistosomum haematobium* (BILHARZ) (= *Bilharzia*), which is now placed in Looss' family, the *SCHISTOSOMIDAE*, all the other man-infesting trematodes—and there are about a dozen of them—belong to the family *FASCIOLIDAE* (RAILL).

II. ANATOMY

Alimentary Canal.—There is no true sucker at the anterior end. The mouth is a simple aperture leading into a pharynx, the walls of which form an almost spherical bulb. The lumen is lined with chitin, and the bulb is separated from the general parenchyma of the body by a basement membrane. Between the basement membrane and the chitinous lining lies a loose tissue crossed by numerous muscle fibres, which mostly run in a radial direction, but a few run circularly.

At first the lumen of the pharynx is compressed from side to side, but after about 30–35 sections from the anterior end the lumen has become depressed from above downwards, and just here are found two short dorsal and ventral valves projecting like tongues into the lumen only directed backwards. They are attached anteriorly and free posteriorly. Behind these valves the lumen becomes diamond-shaped, the long axis being the transverse one, and here the bulb is at its largest and occupies a good deal of the area within the body wall. Its wall is also now divided into an outer and inner layer by a well-marked layer of circular muscles. The inner layer consists largely of radiating muscle fibres. The whole bulb lies somewhat freely in the very loosely vacuolated parenchyma, which seems to form a space around it transversed only by a few sparse threads of protoplasm.

1. *P. Asiat. Soc.*, Bengal, 1876, p. 182.

As we pass into the posterior half of the bulb the diamond-shaped lumen becomes a slightly oval slit whose angles shortly afterwards are turned down, thus forming a crescentic-like space in cross section. At the hinder end of the bulb these turned down corners are cut off from the central lumen and form two lateral diverticula, the pharyngeal pouches (Fig. 7, Pl. IV). The diverticula, although they have their origin in the turned down corners of the lumen, soon come to lie dorso-lateral of the central channel, and this alteration in relative position is caused by the central channel passing toward the ventral surface of the body. The pharyngeal pouches consist of the same kind of loose vacuolated tissue as the bulb, they are very thick-walled and with small lumina.

Behind the bulb the lumen of what may now be called the oesophagus deepens, and in the region of the anterior border of the genital pore, the central portion of the alimentary canal is no longer surrounded by the characteristic tissue of the bulb, though the two dorso-lateral diverticula, which still persist, are. The lumina of these diverticula then become slightly coiled so as to appear twice in one section, and then each of them fades out and disappears altogether. At about the level where the anterior third of the body joins the posterior two-thirds, the oesophagus divides into the two lateral diverticula, and around the λ-shaped lumen at this point is a thick bulb or sheath of muscle fibres mostly circular in their arrangement, though some are radial. Around them is a layer of longitudinal muscles (Figs. 5 and 6, Pl. IV). The lateral diverticula now pass outwards and begin to include between them the reproductive organs. Each diverticulum is flattened sideways and has a considerable dorso-ventral axis. They give off no secondary diverticula, though they are wavy or wrinkled, especially posteriorly, and here also they diminish in size, pass dorsally, and come to an end just about the level of the anterior lip of the great posterior sucker.

It does not seem possible to make out any cells lining any part of the gut. No epithelium is recognizable. The lumen is lined by a deeply-stained layer which looks like mucus, very thin in the pharynx but quite thick in the intestinal diverticula. At the outer surface of this deeply-staining layer, darkly-stained structures, which may be nuclei, are here and there to be seen. The whole rests on a very definite basement membrane, and outside this in the region of the diverticula is a single layer of longitudinal muscles, the whole recalling in appearance the structureless lamella and the muscle-tails of the ectoderm cells lying on it, in a Hydra (Fig. 10, Pl. IV).

The Excretory System.—The excretory pore lies in the middle line above the posterior sucker. It opens into a tube lined with cuticle directly continuous with that which clothes the body. This canal is pushed a little way out of the median line and lies, in the single specimen reduced to sections, a little to the left. Its walls soon thicken, and numerous darkly-stained structures appear in its periphery, there may be nuclei or possibly sections through minute muscle fibres. Passing forward the canal enlarges and forms a spacious vesicle which still lies over the sucker,

spreading over its anterior end ; from this vesicle, secondary canals pass up into the surrounding tissue, these, however, cannot be traced further in sections. The bladder or vesicle narrows again as we pass forward, and by the time the anterior edge of the sucker is reached, it comes to lie between the hindermost ends of the diverticula of the alimentary canal. In front of this the main trunk seemed to divide into two, but beyond this they could not be traced.

The Parenchyma—This packing or ground tissue consists of large cells usually diamond-shaped in section. They are evidently very soft, and have been pulled out into strand-like structures where the cuticle has been elevated. The cells contain a granular-looking protoplasm. The cells underlying the cuticle are much smaller than those of the parenchyma within, the details could not be made out, but amongst and between them are some obvious muscle fibres. Similar muscle fibres lie outside the gut-diverticula, and many such fibres surround the outer parts of the reproductive ducts.

The Reproductive Organs.—There is a genital papilla (Fig. 2, Pl. IV) situated in the middle ventral line about the level where the anterior quarter joins the posterior three-quarters. On this open close together the canal of the cirrus and the metatrema, the vas deferens opening slightly in front of the latter. The whole papilla is but slightly projecting ; its tissue is closer and firmer than the usual body tissue. The distal end of the cirrus canal is muscular for a short space, and seems to have glands opening into it, but it soon gives a bend and opens into a thin-walled vesicle on the ventral surface, the vesicula seminalis, which in the specimen that was cut into sections contained a mass of spermatozoa. The genital papilla is on a level with the lateral diverticula of the oesophagus, but the vesicula seminalis lies beneath the muscular pharynx, just where the alimentary canal is beginning to split into two diverticula. Ventral to it lies the small vagina with muscular walls which, just behind the level of the opening of the cirrus canal into the vesicula seminalis, expands into the thin-walled uterus.

The vesicula seminalis opens into the vas deferens dorsally, and begins to pass backward as a slightly coiled, thick-walled duct. This is still packed with spermatozoa. The thick-walled duct suddenly passes into a thin-walled duct, which is closely coiled and still packed with spermatozoa. The junction of the two is at the level where the uterus begins to pass dorsally ; it continues, however, to lie ventral to the coiled thin-walled portion of the vas deferens. The testes are double, and lie side by side, though one projects further back than the other. They are ventral to the uterus, which for a short space lies between the glands and their ducts. The testes are closely adpressed to one another, and it is just possible that they unite at one point. They open straight into the thin-walled vas deferens. Each testis is deeply lobulated. The glands are packed with sperm morulas in various stages of development, their darkly-stained nuclei giving the tissue a very characteristic appearance.

The metatrema or distal and modified end of the uterus opens close behind the vas deferens: it is a thick, muscular duct which passes backward for a short distance in a straight line. Just in front of the anterior border of the testes it enlarges into the uterus, and this begins to twist and loop, lying between the dorsally-placed vas deferens and the ventrally-placed testes. The uterus contains ova, but not in very great quantities; the eggs are encased in a shell and contain many deeply-staining yolk-granules, but little more can be made out. My measurements for an ovum, which looked unusually large, were 122 μ × 80 μ, but CONYNGHAM gives 130 μ × 75 μ. Undoubtedly the eggs vary in size to a certain extent. The uterus coils a good deal over the testes, and at the posterior end of these glands its lumen enlarges, and it becomes filled with a glairy-looking coagulum in which the ova lie embedded.

The ovary or germarium lies close behind the testes, and rather to the right of the body: it contains minute ova with large nuclei, closely packed together in some places and loosely in others. The whole, like the testes, is ensheathed in a connecting tissue casing. The oviduct leads from the anterior end and curves back above the ovary, it becomes almost immediately surrounded by the shell gland, and may here be called the ootype. Close behind the shell gland the ootype receives the opening of the vitelline duct, and the inner end of Laurer's canal. The shell gland and the ovary come to an end at about the same level as the anterior edge of the posterior sucker. There is a well-marked canal of Laurer which passes almost directly dorsalwards and opens in the dorsal middle line, just in front of the posterior sucker.

The yolk glands are conspicuous, follicular structures, which take no stain, but remain a somewhat dirty-brown colour, somewhat glistening. They extend forward as far as the reproductive pores, and they lie near the edge of the body, ventral to the right and left branches of the alimentary canal. The glands increase in number posteriorly, and in the region of the great sucker are very numerous. Their minute ductules fuse together and gradually unite into right and left ducts that open into the ootype, which is surrounded by the shell gland, and in which the egg is made up. Into the same space opens the duct of the yolk reservoir, which is a coiled receptacle, full of yolk, lying to the left and opposite the ovary.

III. SYSTEMATIC POSITION

The trematode we have to do with has been described by Mr. H. F. CONYNGHAM[1] as a species of the genus *Amphistoma*, which he calls *Amphistoma watsoni*. Dr. F. FISCHOEDER has recently pointed out that the name *Amphistoma* is in reality a synonym of the genus *Strigea*, but the original *Strigea* has since been described as *Hotostomum macrocephalum*, and if *Strigea* is to be revived it must be for that form. Hence Dr. FISCHOEDER proposes to us the name *Paramphistomum* for what we have

1. *B. M. J.*, No. 2,281, Sept. 17, 1904, p. 663.

been used to term *Amphistomum*, and the name *PARAMPHISTOMIDAE*[1] for the family to which they belong. Whether we follow the classification of BRONN's Thierreich or as I propose to do the later classification of FISCHOEDER, it is impossible to class the new human parasite described above as an *Amphistomum*, because that species is characterized, amongst other things, by the absence of the lateral diverticula of the pharynx which form so characteristic a feature of our species. This fact, however, could only be determined by cutting the animal into sections, and therefore escaped the notice of Mr. CONYNGHAM.

FISCHOEDER divides the *PARAMPHISTOMIDAE* of the Mammalia into two sub-families—(i) the *PARAMPHISTOMINAE* with the genera *Paramphistomum*, *Stephanopharynx*, and *Gastrothylax*, all these being devoid of pharyngeal side pouches, and (ii) the *CLADORCHINAE* with the genera *Cladorchis*, *Chiorchis*, *Gastrodiscus*, *Homologaster*, and *Balanorchis*. Of these genns, *Cladorchis* is characterized by having the body not divided into anterior and posterior portions, by having the lateral edges rounded, by having the ventral surface slightly hollowed, and in all these respects our genus agrees with *Cladorchis*, and differs from the other members of the sub-family. I therefore place it in this genus.

CLADORCHIS WATSONI (CONYNGHAM).

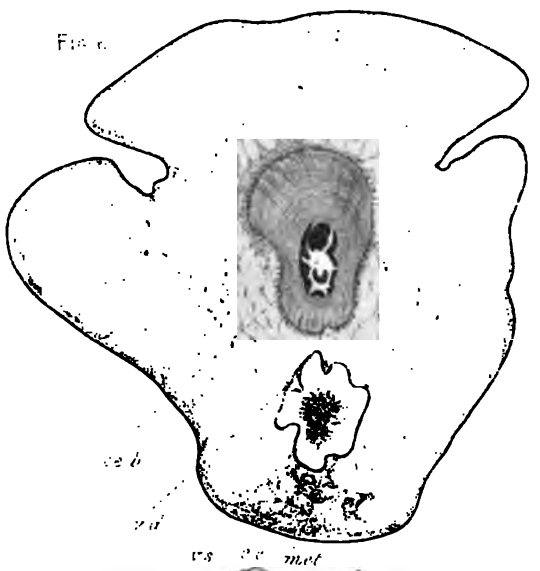
Synonym. *Amphistomum watsoni* (CONYNGHAM).

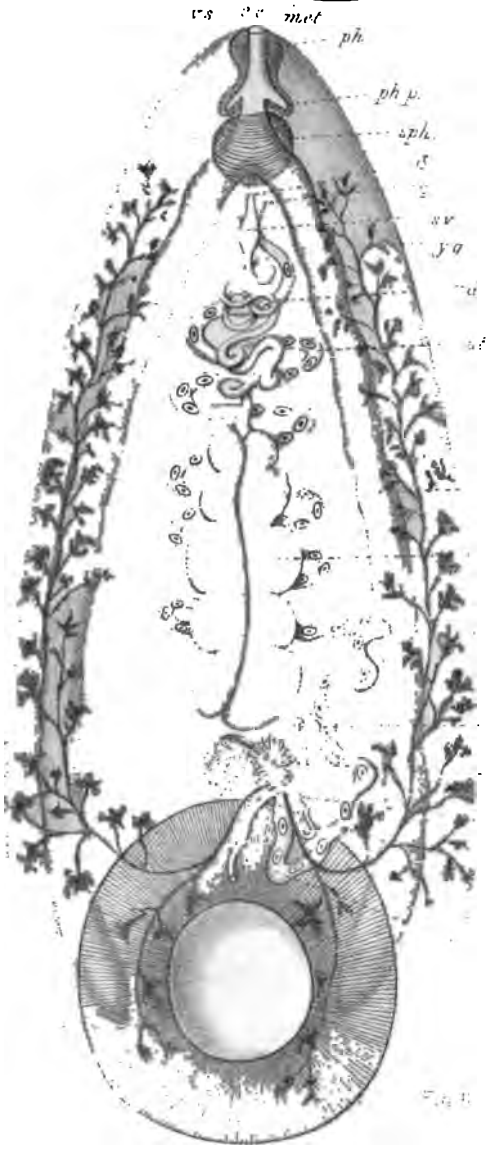
Length, 8-10 mm.; greatest breadth, 4-5 mm., tapering towards the anterior end to about 2·5 mm., and depth about 4 mm.; colour, when fresh, reddish yellow, when preserved, a dirty brown ; when fresh, translucent and gelatinous ; the ventral surface transversely wrinkled, the aperture to the posterior sucker small, but the sucker itself big ; no distinct sucker anteriorly but a well-marked pharyngeal bulb ; the two pharyngeal pouches project beyond the outer limit of the bulb ; circular sphincter round the oesophagus just where it forks ; distinct genital papilla, testis lobed, divided into two, side by side, anterior to ovary ; Laurer's canal straight, opening anteriorly to excretory vesicle in middle line above the posterior sucker ; the latter is very large and vaulted, the ova measure from 122.130 μ × 75-80 μ.

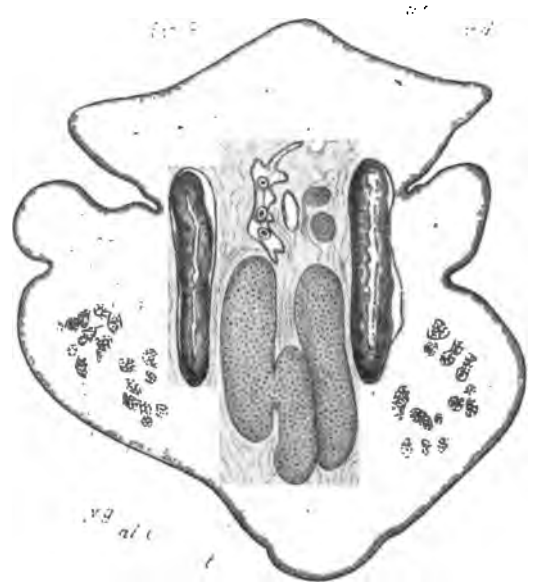
Habitat. *Homo sapiens*, a West African negro, jejunum and duodenum, very few in the large intestines.

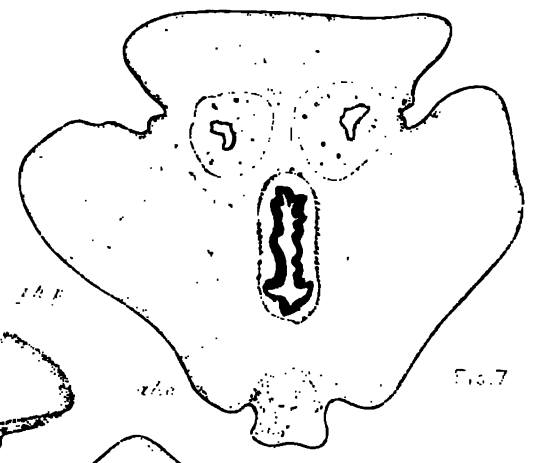
1. *Zool. Jahrb. Syst*, xvii, 1903, p. 485 *v.* also *Zool. Anz.*, 1900, p. 367.

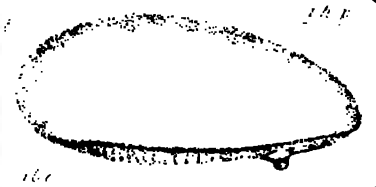
PLATE IV.

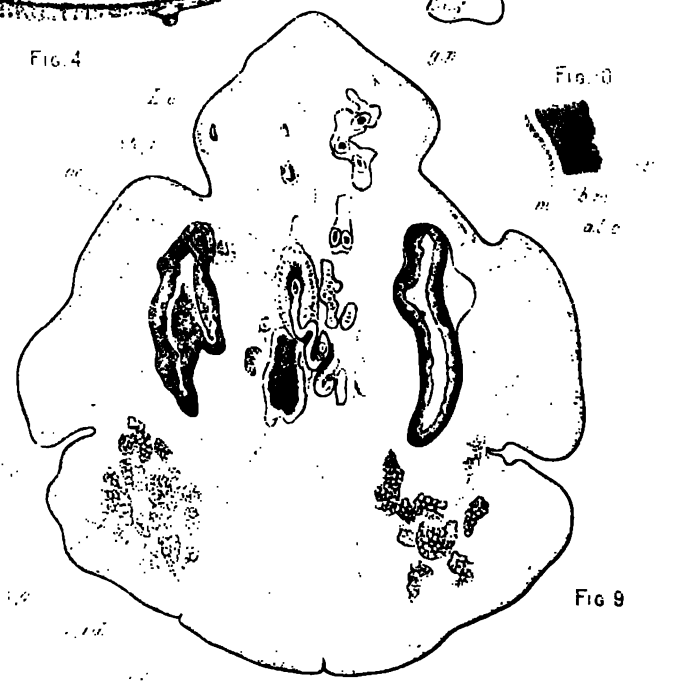












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Explanation of Plate IV

ILLUSTRATING Mr. A. E. SHIPLEY'S ARTICLE ON *CLADORCHIS WATSONI*

Reference Letters

Al.c., alimentary canal ; b.m., basement membrane ; c.c., cirrus canal ; ep., epithelium of gut diverticula ; g.p., genital papilla ; L.c., Laurer's canal ; m., muscles ; met., metabrema ; m.g., in Fig. 5, shell gland ; oe.b., oesophageal bulb ; oo, ootype ; ov., ovary ; ph., pharynx ; ph.p., pharyngeal pouches ; sh.g., shell gland ; sph., sphincter muscle around oesophageal bulb ; s.v., seminal vesicle ; t., testes ; ut., uterus ; v.d., vas deferens, in Fig. 6 it is just leaving ; v.s., the seminal vesicle ; y.g., yolk glands ; y.g.d., duct of yolk glands ; y.r., yolk receptacle.

Fig. 1. Ventral view of *Cladorchis watsoni*, × about 4, showing the genital papilla and the aperture to the posterior sucker.

Fig. 2. The same, natural size.

Fig. 3. Anterior view of the same, showing mouth and genital papilla, × about 4.

Fig. 4. Side view of the same, showing genital papilla, × about 4.

Fig. 5. A diagram showing the disposition of the organs in *Cladorchis watsoni*. The positions of the various organs are distorted so that they may all be shown.

Fig. 6. A transverse section through the region of the oesophageal bulk and seminal vesicle.

Fig. 7. A parallel section anterior to that in Fig. 6, showing the oesophageal pouches and the anterior part of the genital papilla.

Fig. 8. A section through about the middle of the body.

Fig. 9. A section a little in front of the posterior sucker, showing the ootype.

Fig. 10. A portion of the wall of the alimentary canal, showing the external muscle fibres, the basement membrane, and the lining layer with granules or nuclei at its base.

THE INTERMEDIARY HOST OF FILARIA CYPSELI

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THE INTERMEDIARY HOST OF FILARIA CYPSELI
(ANNETT, DUTTON, ELLIOTT)

THE FILARIA OF THE AFRICAN SWIFT CYPSELUS AFFINIS

By J. EVERETT DUTTON, M.B.
WALTER MYERS FELLOW, LIVERPOOL SCHOOL OF TROPICAL MEDICINE

WHILE staying at Bathurst, Gambia, on a mission sent to the colony by the Liverpool School of Tropical Medicine, in 1901, I had opportunities to extend the studies commenced in Nigeria, in 1900, by the members of the Liverpool Malaria Expedition sent to that colony by the Committee of the School.

On my return from Bathurst, lack of time and opportunity prevented me from collecting together the facts acquired on the Gambian Expedition in anything like a concrete form, and, as the Committee of the School deemed it advisable to despatch another expedition to the Gambian colony, I thought it better to delay writing until I had made further studies on the subject of this present paper. During my second visit to the colony no further advance was made; however, in view of its interest, and of the light that may possibly be thrown on the transmission of some of the human filaria, I propose to give here the life-history of this bird filaria as far as I have worked it out.

The swift, *Cypselus affinis*, is a very common bird on the West African coast; in certain places it occurs in enormous numbers. It generally builds its nest near human habitations, in neighbouring palm trees, or on old walls, but more especially under the eaves and on the rafters of bungalows. The nests are built of mud and are lined with grass and a few feathers—often there are three to six nests in close proximity to one another. At night the birds sleep huddled together in their nests, the young birds staying with their parents for some time after they are able to fly; it was an easy matter to obtain birds for examination after they had retired for the night. Generally two to six birds were found in each nest.

Nearly all the birds examined were found to be more or less infested with a small biting louse, which Mr. F. V. THEOBALD[1] very kindly informs me belongs to

(1). DEAR DR. DUTTON.—All I can get out about the lice from swifts in W. Africa, that you sent me, is that they are *Lerothinae*—I cannot find the species, I am sorry to say, and most likely it is new.—FRED. V. THEOBALD.

the sub-family *Leiothinae* ; species uncertain. This parasite is represented in Fig. 10. The louse lives amongst the feathers of the birds, and is generally found on the under-surface of the long feathers of the wing on either side of the quill. When disturbed the parasite moves rapidly among the feathers and, because of this, together with its small size and flat body, it is difficult to capture. Eggs are laid and glued to the under surface of the feathers. On the death of the swift, by placing the body in water in a white dish, the lice come to the surface of the feathers, and can be picked off by means of a dissecting needle. The lice feed on the feathers of the birds and also on small quantities of blood and lymph drawn from their hosts ; blood and bits of feathers can be found in the stomach of both male and female lice on dissection. It was in the bodies of these lice that a further development of the embryo of *F. cypseli* was observed, and it was fortunate that specimens of the young filaria were obtained in all stages of their metamorphosis up to those in which they bear a marked resemblance to their parents.

If we consider for a moment the habits of the swifts, as described above, it is easy to understand how lice, infected with the parasite, can travel from one bird to another through the medium of the feathers, and so spread infection. Though I have examined the nests of the birds most carefully I have never found lice in the dried mud and grass.

Infection among the Gambian Swifts.—The members of the Expedition to Nigeria have shown that the habitat of the embryo of *F. cypseli* is essentially the lymph, they rarely found embryos in the peripheral blood, and never in the heart's blood of the infected birds. It was pointed out that the claws of many of the birds infected were generally found to be swollen, and on careful manipulation, serous fluid, free from blood, could be obtained from these claws, and in the fluid many embryos were usually seen.[1] These facts were fully confirmed at Bathurst.

The method of examining the birds was as follows : the side of one of the claws was pricked once or twice with a fine needle, and then slightly compressed ; from the drop of blood-stained serum exuded a coverslip preparation and a film were made. In infected birds clear serum would generally exude from the punctures without pressure of the claw.

On November 1, eight adult swifts were examined ; three were found to be infected. On December 2, six swifts were examined, two were infected. Two young birds, just fledged, examined, were not found to be infected. On December 23, two adult birds were examined with negative results. These birds were taken from two houses in the centre of the town. After the 23rd of December I was never able to obtain birds for examination ; the dry season was fully advanced ; there was a great scarcity of insects of all sorts ; and for these reasons I judged the swifts had left the locality.

1. *Report of the Malaria Expedition to Nigeria* (1900), Part II, *Filariasis*, Liverpool School of Tropical Medicine.

Filaria cypseli.—The *adult worms* found on dissection of the infected swifts were in all respects similar to those found in Nigeria by the members of the Malaria Expedition,[1] their 'site' was also similar.

Of the three swifts found infected on November 1, parent worms were not found in the first bird examined. In another bird, one immature female and one male parent were found in the subcutaneous tissue behind the occiput; they were not together, the former being coiled up by itself. In the third bird, one female parent was found coiled up under the subcranial tissue on the top of the head. In one of the two birds found infected on December 2, two females (one fully grown) and one male adult were found under the subcranial fascia, near the posterior corner of the right eye. All of the worms were found coiled up together. In the other bird parent worms were not found. It is very probable that many parent worms escaped detection.

The Embryo (Fig. 1).—In one of the infected birds embryos were seen in the lymph taken from the subcutaneous tissue of the back. The embryo is described as follows in the Report of the Expedition to Nigeria[1]:—

'The embryo in the fresh condition as seen in lymph and some blood preparations was 84·7 μ long. The breadth of the sheath of the embryo, 12·78 μ; of the embryo itself inside its sheath, 7·9 μ. When fresh, the embryos exhibited a slow sinuous progressive movement; while inside the sheath they were much more active. The two ends of the worm continually moved about, so that the tips seemed always in contact with the inner surface of the bluntly conical end of the sheath—the ends never being observed retracted from the sheath. This movement of the extremities inside the sheath, which appears a little too short for the embryo, causes the embryo to be thrown into two curves. Ecdysis was not observed. Both extremities of the embryo are bluntly rounded. At the anterior extremity is a short stout conical papilla, from the apex of which projects a short thick spine which is always closely applied to the inner surface of the rounded end of the sheath. There is no prepuce, but a distinct ridge marks off the body from the papilla; neither spine nor papilla was observed to be withdrawn. Under high powers a central line appears to run down from the papilla into the body. The anterior portion of the body of the embryo tapers very slightly. The contents are finely granular, a larger more refractile granule appearing at a point at about a quarter of the length from the posterior end. At this end the worm has a short, rather broad, highly refractile tubercle, which is always in contact with the sheath, and moves from side to side along the concavity of the end of the sheath.

'In fixed and stained preparations, in all of which the embryo is found shrunk in

1. *Report of the Malaria Expedition to Nigeria* (1900), Part II, *Filariasis*, Liverpool School of Tropical Medicine.

various degrees inside the capsule, the length varies from 75 to 84·7 μ. The nuclei of very small cells are evident, but indications of V or other shaped spots are very indefinite and irregular.'

Young Filariae in the Bird Lice.—The following table shows the number of lice taken from the infected birds examined and the number found infected :—

Date	Number of lice examined	No. of lice containing filariae
Nov. 1	5 taken from 3 infected birds	3
Dec. 2	6 „ „ 1 „ bird	0
	6 „ „ 1 „ „	3 (2 ♀, 1 ♂)

The lice were dissected in normal saline solution on a slide ; as a rule the head and mouth parts, the thorax, and the abdomen were examined separately. In the two former portions filariae were never seen. The intestinal canal of the first louse found infected was drawn out in a manner similar to the method by which the 'stomach' of the mosquito is obtained for examination. On examination of the preparation under the microscope large and small filaria were seen free in the saline solution. In all of the first three infected lice, variously sized free filariae were found in their abdominal cavities.

On December 2, as seen from the table, six lice obtained from one infected bird showed no filaria, at least none were found in the abdominal cavity. After this batch was examined, a more minute dissection was made of the walls of the abdomen in the lice taken from the other infected bird, and was rewarded by the discovery of very young forms of filariae in the loose tissue lying beneath the chitinous exoskeleton. The tissue containing these young forms is probably the fat body, as no muscular structure was observed in it.[1] No filariae were seen in the malpighian tubules or in the stomach wall. Altogether over twenty filariae were observed in all stages of development in the fat body and free in the abdominal cavity. In the latter situation very long and active forms were seen, resembling in miniature the parent worms found in the subcutaneous tissue of infected swifts.

From the above material the steps in the metamorphosis of the parasite in the louse can be almost completely worked out. For convenience the following stages will be described :—

Stage I (Fig. 2).—The embryo loses its sheath and its body becomes slightly granular; there is no alteration in length or shape of the worm. The loss of the sheath probably takes place in the stomach of the louse, as small embryos, both with

1. Grassi has observed various stages of the metamorphosis of *F. recondita* in the fat cells of *Pulex serraticeps*, *P. irritans*, *Rhipicephalus siculus* (Koch).

and without sheaths, are seen in the stomach contents. The peculiar tubercle at the posterior end is plainly seen, and by it the small embryo can at once be recognized. The young filaria now show active progressive movements; it is probable that, soon after casting its sheath, the embryo pierces the stomach wall and, finding its way to the fat body, there embeds itself. Length, 84·76 μ; width, 6·5 to 7·5 μ. Length of tail tubercle, 3·26.

Stage II (Fig. 3).—Found in the fat body. When dissected out from the fat body the parasite generally shows little or no movement. The chief feature of this stage is the marked shortening and thickening of the embryo. The tubercle at the posterior end is still present, but appears rather smaller in comparison to the increased width of the parasite. The slight ridge separating the body from the papilla at the anterior end of the body is not visible, and the extreme end is slightly flattened. The body itself is seen to consist of a central, very granular portion, surrounded by a thin hyaline envelope. In some parasites a minute, lateral tubercle may be seen situated at the side of the body, at the junction of the anterior and middle thirds, also another slight elevation of the cuticle may be seen near the posterior end.

The following are some measurements of embryos in this stage of their metamorphosis, seen in fresh preparations (normal saline):—

Total length	Central width	Length of tail cuticle
81·50 μ	9·78 μ	3·26 μ
58·68	16·30	3·26
75·24	18·56	3 26
94·80	19·50	3·26

Fig. 4 represents the embryo with a much flattened anterior extremity, and with evidences of a mouth opening and lips—this appearance has only been seen in one specimen, but it is very probable that during the end of this stage development of a mouth opening takes place as described by MANSON[1] in the metamorphosis of *F. bancrofti*. The embryo, measuring 94·8 μ, is represented in Fig. 5. It shows a slight transition from this to the next stage to be described; the body is differentiated into outer and inner layers; the portion of the anus is clearly distinguished, and commencing striation of the body can be seen.

Stage III.—Stage of elongation and differentiation of the body protoplasm (Fig. 6). In this stage of its metamorphosis the young filaria is found greatly increased in length, which now progresses much more rapidly than the still increasing

1. Manson. *Transactions of the Linnean Society*, 1884, p. 367.

width of the organism. The body of the parasite is markedly striated, the margin in profile showing a double contour, due to the presence of a very fine enclosing membrane. This membrane is very well seen at the anterior and posterior extremities. The tail tubercle is still present but only measures about 2μ in length, and appears very minute in proportion to the body of the organism. Besides these external structures, the position of the anal aperture is easily made out, situated a little way from the tail end on a slight prominence, generally there is seen extruded from the aperture a large highly refractile granule. In some organisms a slight papilla is seen some little way from the head end on the same border of the parasite as the anal tubercle. The internal structure of the parasite shews very clearly the differentiation mentioned in the previous stage of its metamorphosis; the outer layer consists of probably two or more layers of cells, the innermost being formed of cubical shaped cells have rather large nuclei (See Fig. 6). The internal granular layer is seen to have become developed into what is evidently the future alimentary canal. This commences at the anterior end of the organism, at a small terminal papilla, as a narrow tube piercing the outer cuticle and continuing on as a comparatively thick column of cells running down the centre of the body. There is an obvious thickening in this column of cells at about one-third the length of the worm from the anterior end. Opposite the anus there is again a large thickening of this cell column, in which highly refractile areas are generally seen. The column of cells now narrows again to form a rather wide tube ending on the anal tubercle. In some parasites this internal cell layer is easily seen to be in reality a cellular tube running down the length of the body. Between the cuticular and alimentary cell layers a space is generally seen—the body cavity. Opposite the anterior lateral papilla a highly refractile rounded area of small cells is observed in some parasites (Fig. 7). It is probable that from these cells the future uterus and ovary or spermatic canal, as the case may be, arises. A little anterior to the genital papilla in some specimens a band of small cells can be made out crossing obliquely the anterior part of the intestinal canal, this band represents the nerve collar (Fig. 7). In this stage the worm is still in the fat body, it shows slight lateral movements when dissected out.

The following are some measurements at this stage :—

Total length	Length to nerve collar	Length to genital papilla	Length to anus	Central width	Width at nerve collar	Width at genital papilla	Width at anus
143·44 μ	Not distinct	—	—	22·82 μ	—	—	—
133·66	„	—	—	29·34	—	—	—
189·06	27·71	Not seen	169·52	25·34	29·34	—	26·08
247·76	35·86	65·2	235·76	32·6	29·34	32·6	26·08

Stage IV. (Fig. 7).—The parasite still increases in length, the width of the body remaining stationary. The anterior and posterior ends instead of being similarly bluntly rounded off show some differentiation. The anterior extremity of the parasite has become bluntly cone shaped, similar to the adult parent worm. The posterior is rounded and slightly flattened at its extremity, it has now lost its tubercle. The fine sheath enclosing the embryo is now more marked, there is a distinct space between it and the body of the worm, at the posterior end it forms a tag-like process which represents the covering of the posterior tubercle. The worm has now lost its markedly striated appearance. In the interior of the worm the alimentary canal is now seen differentiated off into pharynx and oesophagus, ending at a bulb-like thickening and intestinal tract. It does not occupy the whole width of the body cavity as in the last stage, and is seen to be moveable from side to side in the fresh state. The other structures mentioned in the previous stage are still more plainly seen. If the worm is dissected out from the fat body—now an easy matter, as the parasite generally becomes free in the process of dissection of the abdomen of the louse—active contractile movements of the parasite are seen.

The following measurements were taken of one parasite in this stage directly after movements had slowed down in two per cent. formal in saline solution :—

Total length	Length to genital papilla	Length to anus	Central width	Width at genital papilla	Width at anus
326 μ	71·72 μ	312·96 μ	29·34 μ	26·08 μ	22·82 μ

The pharynx and oesophagus up to the bulk-like thickening measured in this worm 162 μ. In this and the next stage of metamorphosis of the parasite, I believe it possible to distinguish the future male and female adult filaria. Thus the future male parasites may be distinguished as follows : The anal opening is situated at a greater distance from the posterior extremity of the parasite, and the end prominence is more marked ; behind it there is a marked constriction in the body of the parasite. When placed in normal saline solution the tail end tends to curl in. There is no genital papilla near the head representing the future vaginal opening. The parasite is smaller than those which have a genital papilla.

Stage V (Fig. 8).—In this, the last stage so far seen in the development of the filaria, the worm is found free in the body-cavity of the louse ; it shows actively progressive and lively movements when placed in normal saline solution. In shape it is exactly like the adult parent worms, except that there is a slight constriction at the tail end, and the position of the anus is not quite subterminal.

Contrasting it with the previous stage, the parasite shows a further increase in

length with a slight diminution in width, and structures which could be made out previously are not so obvious, the young filaria being now rather opaque ; still the genital papilla, the anus, and alimentary canal can be made out. The anal opening is not so widely open as in the previous stages. No further development of the reproductive system has been observed.

The worm has now lost the fine sheath enclosing it, this sheath is probably cast off when the filaria issues from the fat body.[1] Situated at the centre of the rounded posterior end of the parasite is a small thorn-like projection, and around it, some little distance away, are three or four similar spines, probably corresponding to the papilla at the tail of the *F. bancrofti* in the last stages of its development in the mosquito.

The following are measurements of two worms about this stage :—

Total length	Length to genital papilla	Length to anus	Central width	Width at genital papilla
368·6 μ	60·3 μ	—	26·08 μ	26·08 μ
612·88	Not seen	—	22·82	—

The young *F. cypseli* has thus been traced to a very advanced stage in its development ; indeed, it is very probable that after Stage V no further development takes place in the body of the louse. The worm now resembles the parent parasite in shape, its movements are very active, and it corresponds in its development to the last stage seen in the metamorphosis of other filariae, viz., *F. bancrofti* and *F. immitis*. For these reasons also I think it is justifiable to conclude that the louse (*sp. incert*) is the intermediate host for this filaria, though actual proof, *i.e.*, by infecting birds by means of lice, is wanting. Granting that Stage V is the last in the metamorphosis of *F. cypseli* before its transference to its definite host, the swift, it has yet to be ascertained how this transference takes place. Two possible ways suggest themselves.

A. The birds may eat the infected lice ; the young filaria escaping from the bodies of the lice in the process of digestion reach the tissues of their future host, via the stomach.

B. The now active filaria may travel through the tissues of the louse and, reaching the proboscis, be transferred to its definitive host when the louse pierces the skin of the swift. *Filaria immitis* is transferred from dog to dog in a similar manner

1. Manson believes that while the young *F. bancrofti* are in the muscles of the mosquito they are surrounded by a fine sheath, which is shed before the worm reaches its final stage of metamorphosis in that insect. That such a sheath is present can be beautifully demonstrated in specimens of the mosquito in *F. bancrofti*, about the thirteenth to fifteenth day of development, if torn across in the process of dissection.

PLATE V

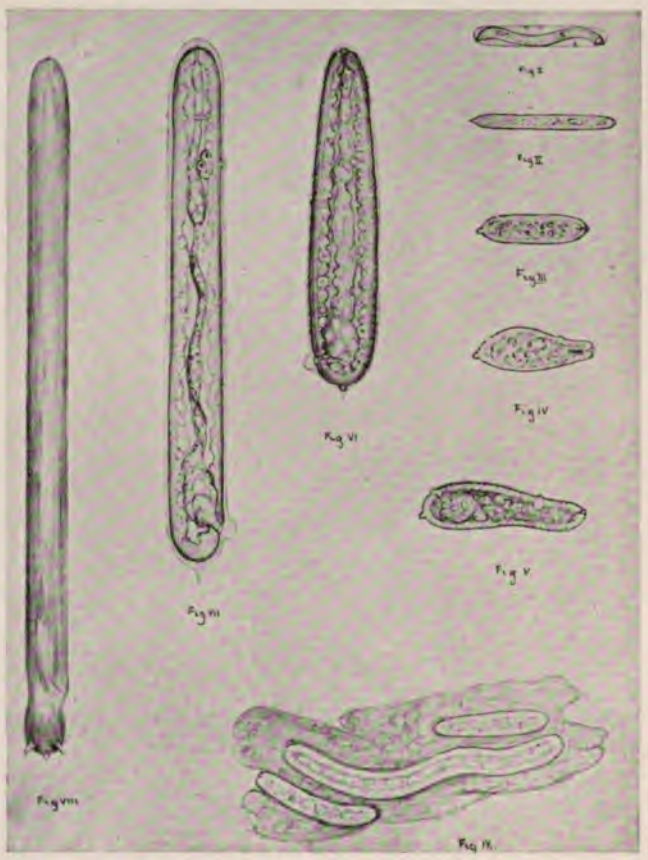

Fig. x

through the agency of *Anopheles maculipennis*, and it is probable that *F. bancrofti* is transmitted in a similar way from man to man. It is quite possible that infection of the swifts may take place by both of the above methods.

The points of interest associated with this filaria are :—

1. Up to the present it has only been found in one species of bird, *Cypselus affinis*, while other African bird filariae are generally encountered in many different species.
2. The habitat of the embryo is essentially the lymph.
3. The louse which acts as its intermediate host, as a general rule, belongs to a family, the members of which usually, though not always, are parasitic on one species of bird only.
4. This louse does not exclusively feed on feathers, but sucks up blood, and probably to a greater extent lymph.
5. The metamorphosis of the parasites is very similar to that of previously known filariae.

In conclusion, I must thank Dr. H. E. Annett for the kind interest he has shown in this study and for the help and suggestions he has given me.

EXPLANATION OF PLATE

Semi-Diagrammatic

Fig. i. Embryo of *F. cypseli* as seen in lymph of swift (× 300).
Fig. ii. First stage in development in body of louse (× 300).
Fig iii. Second stage „ „ „ (× 300).
Fig. iv. Showing development of mouth opening (× 300).
Fig. v. Showing differentiation of body into inner and outer layer and commencing striation.
Fig. vi. Stage III. Optical median section of parasite (× 300).
Fig. vii. Stage IV. „ „ „ „
Fig. viii. Stage V. „ „ „ „
Fig. xi. Embryos seen in lining tissue of abdominal cavity of louse (× 150).
Fig. x. Intermediary host of *Filaria cypseli*, sub-family *Leiothinae* sp. incert.

AN EXPERIMENTAL STUDY OF THE PHYSICAL CHEMISTRY OF ANAESTHESIA IN RELATIONSHIP TO ITS CAUSATION

AN EXPERIMENTAL STUDY OF THE PHYSICAL CHEMISTRY OF ANAESTHESIA IN RELATIONSHIP TO ITS CAUSATION[1]

By BENJAMIN MOORE, M.A., D.Sc.
JOHNSTON PROFESSOR OF BIO-CHEMISTRY, UNIVERSITY OF LIVERPOOL
AND
HERBERT E. ROAF, M.B., Toronto
JOHNSTON COLONIAL FELLOW, UNIVERSITY OF LIVERPOOL

THE production of insensibility by the action of drugs was a source of wonder and fanciful speculation to the ancients long before the commencement of the modern history of anaesthesia, and frequently forms the theme of poet, dramatist, or novelist.

It is hence not to be wondered at that in more recent times it has powerfully stirred the imagination and the interest of scientific workers who have sought, by theory and experiment, to account for the profound effects produced upon living structures by such chemically indifferent and inert substances as are most anaesthetics.

The first fact which appealed to the earlier observers was the similarity between anaesthesia and sleep, and experimentation was accordingly directed towards the discovery of points of similarity between the two conditions. The variation of blood supply to the brain by the varying calibre of the small arterioles was a common explanation early offered for both sleep and anaesthesia.

Durham,[2] in 1860, demonstrated by direct observation made after trephining, that the brain surface was paler and hence more anaemic when sleep came on, and flushed again when awakening occurred, and so disposed of the older theory which had never been supported by experiment that sleep was due to a hyperaemia of the brain.

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1. The experiments here recorded have formed the basis of a previous communication to the Royal Society, published in *Proc. Roy. Soc.*, Vol. 73 (1904), p. 382. We have to thank the Royal Society for permission for their republication with the accompanying illustrations. The expenses connected with the research have been paid by a grant allotted for the purpose from the Government Grant to the Royal Society.

2. Arthur E. Durham, *The Physiology of Sleep*, Guy's Hospital Reports, Third Series, Vol. 6 (1860), p. 149.

Following closely upon this observation of DURHAM, BEDFORD-BROWN[1] demonstrated in the same year by observations on a patient having the brain surface exposed, as the result of accident, that the induction of anaesthesia caused a primary hyperaemia which soon passed off and was succeeded by anaemia.

Contradictory observations followed on this by various observers, some of whom found hyperaemia and some anaemia during anaesthesia, but a critical research by CLAUDE BERNARD[2] demonstrated the accuracy of BEDFORD-BROWN's observations, and there is now little doubt that the observations of hyperaemia were due to the presence of inflammation, the result of unskilful trephining, and not to the action of the anaesthetic. But while it may be taken as established, from these observations, that both sleep and anaesthesia are accompanied by anaemia of the brain, it is obvious for various reasons that this can only be a secondary factor or accompaniment, and not the primary cause of either condition.

In the first place, anaesthesia can be produced in organisms in which there is no blood supply and even no nerve cells, for anaesthesia is a condition which can be impressed upon any living cell no matter how lowly its organization. Hence the primary action must be directly upon some constituent of the cell and not solely upon something outside, although in the case of the higher cells, such as the cortical cell of the mammal, interference with its nutrition by alteration in blood supply may act as such a cause and produce anaesthesia. There is no doubt whatever that suddenly produced anaemia of the brain, such as occurs, for example, by double compression of the carotids, by vagus action upon the heart, or by the sudden hydrostatic fall in cerebral blood pressure caused in debilitated individuals by assuming the standing position, gives rise at once to an anaesthetic effect and to sudden loss of consciousness. But here again the cause of the anaesthesia is something of a chemical nature suddenly occurring in the nerve cells, the result of the stoppage or alteration in the cerebral circulation, and not a merely mechanical effect produced by the fall of pressure.

Secondly, although the lessened blood flow in the brain may assist in maintaining sleep or anaesthesia, by lessening the metabolic changes in the nerve cells, it is quantitatively too slight, acting alone, to produce the whole effect in this manner.

Thirdly, even admitting that a part of the effect in both sleep and anaesthesia may be due to altered blood supply, there remains to be sought out the cause of the altered blood supply. This, whether it be a direct effect upon the musculature of the blood vessels or be due to an indirect effect through the vasomotor system, must in the end arise from some chemical effect of the anaesthetic upon muscle cell or nerve cell.

Hence, while there is probably similarity in mode of causation of sleep (including coma) and anaesthesia, such effects as variations in blood supply can only be set

1. *American Journal of Medical Science*, Oct. (1860).
2. Claude Bernard, *Leçons sur les Anaesthèsiques* (1875), p. 117.

down as secondary factors, due also to the same primary cause as directly acting upon the nerve centres, produces the unconsciousness of sleep, of coma, or of anaesthesia.

It is clear then that we must turn to a consideration of the effects of the anaesthetic upon such constituents as are present in the cell, and as a preface to the consideration of the various theories which have been put forward, it may be well to point out that although anaesthesia in mammals means the abolition of consciousness, and the accompanying incapacity for the production of sensation following peripheral stimulation of nerve endings, yet this effect only differs in degree from other effects which appear as the concentration of the anaesthetizing agent is gradually increased. The cortical nerve cells pass first under the influence of the anaesthetic, while other portions of the nervous mechanism are still active and amenable to stimulation. For example, the reflex nervous mechanisms throughout the medulla and cord are still active, such as those which regulate the respiratory and cardiac rhythms, peristalsis, the condition of the vasomotor system, and the sphincters throughout the body, although the degree of activity is, in most instances, depressed.

If the concentration of the anaesthetic be increased, however, these remaining portions of the nervous system also pass under the action of the anaesthetic, and death of the mammal ensues, usually from stoppage of the action of the nerve cells responsible for the continuance of the respiratory rhythm.

Nor is it the cells of the nervous system alone which are affected by anaesthetics, for at increasing concentrations isolated mammalian tissues are affected, and their activities inhibited and finally stopped, as has been demonstrated experimentally for nerve fibres, for cardiac and skeletal muscle, and for ciliated cells.

The same effects are demonstrable not only for mammalian tissues, but also in all living cells of whatever source, whether of vertebrate or invertebrate, of animal or plant, of the lowliest unicellular organism or of the highest mammal.

This result is of importance, as clearly showing that the effect of the anaesthetic throughout the whole range of living cells is produced in some common fashion upon a constituent which is uniformly present in every living cell, and is not something peculiar to the nerve cell, nor explicable by some morphological arrangement present in the nerve cell and not present in other cells.

The result teaches, for example, that no faith can be placed in a mechanical theory of anaesthesia, such as the withdrawal or protusion of the dendrites of the nerve cell, for this would yield no explanation of the similar action of anaesthetics upon other types of cell.

The universality of the action of anaesthetics upon all living structures further narrows the field of search for the substance upon which the anaesthetic acts, since any occasional cell constituent may at once be removed from consideration, and only those left which are invariably present.

When this view has been taken, the material which at once occurs to the mind is that which is most intimately connected with the activity of the cell, and is always present, namely, the cell-protoplasm, or, since we are considering the matter from the chemical point of view, the organized proteids which go to build up the cell.

In addition to the proteids, there is another substance, or rather group of substances, widely distributed throughout the world of living cells, namely, the lecithins and allied bodies which have recently received the name of 'cell lipöids.'

If by the term 'cell lipöids' is to be understood anything soluble in ether, so including bodies so widely dissimilar in nature as neutral fats, cholestearin, and lecithin, it must be admitted that all cells contain 'cell lipöids,' although in many cases, even with this wide and somewhat meaningless definition, the amount present, compared to the proteid and other organic constituents, is infinitesimally small in many types of cell. But if the term is taken to mean lecithin and its compounds only then there exists no experimental proof that 'lipöids or lecithins' are present in appreciable quantity in every cell, and exception must be taken to the important position which has recently been assigned to these so-called lipöids in governing such a fundamentally important process as selective absorption, or as agents in producing anaesthesia by virtue of their high solvent power for anaesthetics.[1]

The earliest experiments which we have been able to trace regarding the action of chloroform and other anaesthetics upon tissues, other than those of the nervous system, have reference to an action of chloroform upon the skeletal musculature which was noticed soon after the introduction of anaesthetics, and attracted the attention of a number of observers. The experiments were made upon the effects produced by injection of chloroform into the arteries supplying the limbs, and the results obtained were a strong contraction of all the muscles, which, with larger quantities, remained permanent, the muscles becoming opaque and stiff, as in the case of heat rigor, or *rigor mortis*.

Although the experiments were carried out with quantities of chloroform greater than are required for anaesthetic purposes, they demonstrate an action upon the muscle substance which is interesting from the point of view of the experiments which we have to describe later in this paper.

The first observer who appears to have worked upon the subject was Coze,[2] who found that after injection of a few drops of pure chloroform into the crural artery of a rabbit, there followed immediately a muscular contraction so pronounced that the muscles appeared to have acquired the hardness of wood. The rigor only slowly disappeared if the animal survived the experiment, but in the end the voluntary movements of the limb became partially restored. Similar rigor was induced by injection of ether, but larger quantities were required to produce the effect.

1. Overton, *Vierteljahrsch. d. Naturf. Gesellsch. in Zurich* (1899), Bd. xliv, S. 88-135; *Studien über die Narkose, Jena*, Gustav Fischer (1901).

2. *Comptes rendus de l'Acad.*, xxviii, 1849, p. 534.

BROWN SEQUARD,[1] in 1853, confirmed COZE's observations, and added that circulation of fresh arterial blood through a limb which had been rigored by chloroform caused a return of excitability even after a period of two to ten days.

KÜSSMAUL[2] independently repeated and confirmed COZE's work, and was first to point out that the condition was one allied to rigor and not to tetanus ; he also showed that, although chloroform caused precipitation in fresh extracts of muscle, the main action of the anaesthetic was upon the substance of the muscle cell. KÜSSMAUL was unable to repeat BROWN-SEQUARD's observation as to recovery of irritability after prolonged action of chloroform.

RANKE[3] found that frog's muscle after the long-continued action of chloroform vapour upon the animals became rigid, and further showed that this was, in all probability, due to a coagulation of the myosin, as he observed a turbidity in clear solutions of the muscle proteid after subjecting to chloroform vapour for a period of three quarters of an hour. RANKE also obtained a similar turbidity, but in lessened degree by the action of the vapours of alcohol, ether, and amyl alcohol upon 'myosin' solutions.

BUCHHEIM and EISENMEYER[4] later subjected frogs to saturated chloroform vapour for an hour, and failed to to get RANKE's muscular rigidity. They found that under such circumstances all movements and the respiration stopped, and the frogs appeared to be dead. The muscle curves taken from the muscles after this treatment showed a short latent period and a normal period of contraction, but the relaxation was veratria-like, and only approached completion after twenty-five to thirty revolutions of the cylinder.

CLAUDE BERNARD in his *Leçons sur les anæsthesiques*, published in 1875, recurred to these experiments on the rigidity and semi-coagulation of skeletal muscle when subjected to the action of chloroform vapour, and suggested that an analogous semi-coagulation probably occurs in nerve cells, and that anaesthesia may be brought about as a result of such a coagulation which may not be definite and fixed, that is to say, in which the cell substance can return structurally to its primitive state after elimination of the toxic agent.

A similar view was expressed by BINZ,[5] who stated that sections of cerebral cortex placed in the one per cent. solution of hydrochlorate of morphia soon showed a cloudy appearance, and fine granules appeared in the nuclei ; the protoplasm also became granular. The stage at which the cell-protoplasm was merely cloudy, and not discretely granular, could be recovered from by washing away the morphia, but, when once the granules appeared, they could not be made to disappear again. Similar

1. Canstatt's *Jahresberichte*, 1853, s. 199. *Experimental Researches Applied to Physiology and Pathology*, New York, 1853.
2. *Arch f. Path. Anat.*, Bd. xiii, 1857, S. 289.
3. Buckhner's *Repertorium*, Bd. xvi, S. 374.
4. Eckhardt's *Beiträge* (1869).
5. *Vorlesungen uber Pharmaklogie*, S. 175.

results were obtained by exposing cortical nerve-cells to vapour of chloroform, or to solution of chloral hydrate.

Neither BINZ nor BERNARD showed that there was any precipitation or semi-coagulation at or near the concentrations which correspond to anaesthesia, nor were the optical methods used capable of demonstrating effects upon the protoplasm short of precipitation.

DUBOIS[1] advanced the theory that anaesthetics produce their effect by a partial dehydration of the protoplasm, by increasing the tension of dissociation between the water and the tissues, as he put it, so that the latter part with a portion of their water, and so tend to approach the dormant condition of dried seeds and other organisms in the dehydrated condition. DUBOIS quotes an experiment in which plant tissues on being subjected to the action of saturated chloroform vapour exuded water, and compares the effect to that produced by cold. There is, however, nothing convincing in the experiments, and the transudation of water is more probably due to injury of the epidermal cells than to any physical action of the anaesthetic directly upon the protoplasm.

SCHMIEDEBERG,[2] in his *Grundriss der Pharmakologie*, states that chloroform retards the passage of the oxygen from the oxyhaemoglobin to easily oxidizable substances and forms a peculiar compound with the haemoglobin, but that these changes cannot be proved to take place in the living body.

Turning from the theories which drive anaesthesia from an action of the anaesthetic upon the cell protoplasm to those which, inspired by the large percentage of bodies soluble in ether which the nervous system contains, look for an explanation to some effect upon the cell lipöids, we find the view advanced by BIBRA and HARLESS[3] soon after the discovery of the anaesthetizing action of chloroform and ether that these anaesthetics owe their activity to their solvent power for the fatty and allied constituents of the nerve cell.

These authors supposed that the anaesthesia was produced because the anaesthetic dissolved out a portion of the lipöid substances, and the experimental evidence on which they based this hypothesis was that after repeated anaesthetization by ether, analysis showed that the ethereal extractives of the brain decreased in amount, while the amount of ethereal extractives in the liver increased.

It is, however, most probable that this result was due to an experimental error, for if there were any actual removal of bodies soluble in ether from the nerve cell, it is difficult (1) to see how the removal could be effected by such small quantities of anaesthetic as are required for the induction of anaesthesia acting merely in dilute solution in the plasma; (2) to see how removal of a small fraction of the total lipöid could produce anaesthesia; and (3) to explain if the removal of this small fraction of lipöid does

1 *Anaesthésie physiologique*, 1894, p. 15.

2. Quoted from the authorized translation by Thomas Dixon, M.B., Young J. Pentland, Edinburgh, 1887.

3. Quoted from Overten, *Studien über die Narkose*, S. 50.

produce anaesthesia, how the anaesthesia can be so quickly recovered from when administration is interrupted ; for the hypothesis presents no mechanism for the rapid restoration of the materials supposed to be removed during anaesthetization. The last argument also disposes effectually of any theory whatever which depends upon a removal of any permanent element of the cell as a cause of anaesthesia.

A somewhat reversed theory to that just discussed which has lately gained much support was put forward independently and almost simultaneously by HANS MEYER[1] and E. OVERTON.[2]

Instead of ascribing the action of anaesthetics, such as chloroform and ether,[3] to the solvent action of the anaesthetic upon the lipöids of the cell, the basis of the theory of MEYER and OVERTON is that the cell lipöids dissolve or take up physically the anaesthetics, by which process the physical properties of the lipöid are altered, and in some manner anaesthesia is induced as a result of this alteration in the lipöids.

The authors leave the manner in which this supposed physical alteration in the properties of the lipöids, by the taking up of the anaesthetic, brings about anaesthesia an open question. OVERTON suggests that the lipöids are either unable to carry out, in this altered condition, their own functions within the cell, or that the new physical conditions of the lipöids acts as a disturbing influence upon the functions of other constituents of the cell.

In regard to the nerve cell, OVERTON further throws out the surmise that the alteration in the condition of the lipöids by the anaesthetic may increase the physiological resistance to the passage of the stimulus both within the cell, and between one cell and another, and also that the activity of the cell to respond when a stimulus reaches it may be lessened by the presence of the anaesthetic in the lipöid.

Although a large number of substances have been examined by MEYER and by OVERTON from the point of view of the relationship of their strength as anaesthetics to their relative solubility in water and in olive oil, and it has been demonstrated *roughly* that the relationship exists, that the less soluble any given substance is in water, and the more soluble it is in oil, the more powerful it is as an anaesthetic. Yet, it may be pointed out, that this by no means settles the matter, for there are other substances in the cell than the lipöids, and it must also be shown, before the argument gathers any weight, that a similar physical property does not belong to any of these, that there are no other substances in the cell which have similar affinities along parallel lines for taking up the anaesthetics, and which may be more intimately connected with the activities of the cell than the lipöid constituents.

It is clear that if the anaesthesia be due to any substance whatever in the cell,

1. Sitzungsber, *d. Gesellsch, z. Beförderung d. ges. Naturwissen*, Marburg, 1899. *Arch. f. exper. Path. u. Pharm.* Bd. X C ii, S. 109 ; see also F. Baum. *Ibid.* S. 119.

2. Overton, *Studien uber die Narkose*, G. Fischer, Jene, 1901.

3. Anaesthetics of this class are designated as indifferent anaesthetics by Overton in contra-distinction to basic anaesthetics, which include such of the alkaloidal anaesthetics as possess basic properties. According to this observer the action of the latter class is different in origin, and probably due to interaction with the cell proteids.

combining physically or chemically (in unstable combination) with the anaesthetic, that the concentration of the anaesthetic in that substance of the cell must vary inversely as the solubility of the anaesthetic in water, because the anaesthetic distributes itself always according to its relative solubilities in the two media between which it is distributed.

This, we maintain, is all that is shown by the experiments of MEYER and OVERTON, who have not experimented with the lipöids, but with olive oil, to which no one attributes the production of anaesthesia, and we regard it as dangerous to transfer the results of experiments made with olive oil and water to lecithin or other lipöids, and water[1].

Again, determinations have not been made, except in a few cases, of the coefficient of distribution of the anaesthetic between even olive oil and water, but instead, only rough determinations of the maximum solubilities in these two media. Also, one meets in, by far, the greater number of OVERTON's experiments with the statement that the anaesthetic, under experiment, is soluble in all proportions in one or other of the two solvents, usually the olive oil. In fact, throughout whole tables of comparison of different anaesthetics belonging to the same organic group, it is observable that the whole of the substances tested are soluble in olive oil in all proportions, and hence, there is no means of knowing what influence relative solubilities in olive oil possess since such relative solubilities do not exist. Under such circumstances it is impossible to tell how the coefficient of distribution is varying, for, of course, the fact that an anaesthetic is soluble in all proportions in olive oil, and has only a low solubility in water, does not indicate that the coefficient of distribution between olive oil and water is infinity. For example, quoting the figures given by OVERTON[2] for two cases, ether is soluble in all proportions in olive oil, and dissolves to the extent of 6·6 per cent. in water, yet its coefficient of distribution between oil and water is only 4·5 ; chloroform is soluble in all proportions in olive oil, and in water to 0·72 per cent., yet its coefficient is only 30—33.

The observation that the anaesthetizing power of indifferent organic substances such as chloroform and ether vary inversely as their solubility in water had already been made by RICHÉT, and is referred to by OVERTON[3] as *Richet's rule* ; but RICHET thought the explanation a different one, and ascribed the action to the fact that such substances being but feebly soluble in water, did not normally enter into the cell or act upon it, and hence exerted a toxic action when they did enter. This explanation,

1. The explanation given for the use of olive oil, instead of the lipöids, or lecithin, is, that the latter are so difficult to isolate in sufficient quantity for the experimental determination of the coefficients of distribution ; but an ethereal extract of brain can easily be obtained in large quantities, and lecithin can now be isolated in large quantity, and with, comparatively, little expense from either brain or egg yolk (see paper succeeding this in the present volume, p. 201). A real difficulty in determining these coefficients in the case of lecithin or the lipöids, lies in the physical properties of these bodies, which are not fluids, but plastic solids, and hence it is not possible to bring a mixture of these and water into equilibrium with such solvents as chloroform, etc., and then to determine the fractions of the chloroform, etc., in the lecithin, or lipöids, and in the water respectively. The difficulty is still further increased by the swelling up and suspension of the lipöids, or lecithin, in the water.

2. Overton, *Studien uber die Narkose*, SS. 96 and 100.

3. *Ibid.*, S. 45

however, does nothing towards elucidating what happens when these substances do enter the cell, nor is there any proof that diffusion into the cell is difficult for such substances, but rather the reverse is true, for the facts of the rapid onset and recovery from anaesthesia show that they enter and leave the cell quite freely and rapidly.

With regard to the statement that in such a large number of cases the anaesthetic is not only less soluble in water but also more soluble in olive oil, it may be pointed out, that in the vast majority of the substances experimented with the substances are soluble in all proportions in olive oil, and hence no comparison is possible. Secondly, it is a general rule in the case of all organic substances of the classes under consideration, that the less soluble they are in water the more soluble they are in organic substances, which, like themselves, are insoluble in water.

For example, if a given organic substance has a low solubility in water and a high solubility in, say, ether, in the great majority of cases it will also have a high solubility in chloroform, benzol, xylol, toluol, and the oils and fats, viz., in substances which have themselves a very sparing solubility in water.

Finally, it may be mentioned that the lipöid theory does not at all fit the experimental facts in the case of the basic anaesthetics[1], or narcotics, which belong to the class of bodies known as alkaloids, such, for example, as coniin, nicotin, and morphia. For this class of compounds, Overton[2] admits the existence of combinations, probably of the nature of salts between the basic anaesthetic and the tissue proteids, and it is strange that this should not have suggested determinations of the action of proteids upon the so-called 'indifferent' anaesthetics, which would have at once demonstrated that these form similar compounds, as is shown by the experiments detailed below.

It hence becomes an advantage of the theory which refers the causation of anaesthesia to the formation of unstable combinations between the anaesthetic and the cell protoplasm, that it furnishes a common explanation of the action of all types of anaesthetic whether basic or indifferent.

A basis is also provided for an explanation of the difference in rate of recovery from the anaesthesia in the case of the different anaesthetics, and for the selective action of certain anaesthetics. For it is obvious that such factors will have an influence as the stability and dissociation pressure of the combination between the anaesthetic and the protoplasm, as well as the rate of diffusion into and out of the cell, in determining the rate of intake and output of the anaesthetic, and hence of the onset and subsidence of the anaesthesia. Further, there must exist differences in the composition and state of aggregation of the proteid molecules going to build up the protoplasm in the case of different classes of cells, and hence facility or the reverse for combination with different chemical compounds or anaesthetics must exist, so that one alkaloid will accordingly pick out and combine with the organized proteid of one type of cell,

1. There are also certain exceptions in the case of the so-called indifferent anaesthetics.

2. *Studien uber die Narkose*, S. 160, et seq.

leaving others free, while another anaesthetic or alkaloid will form combinations in a different type of cell and so produce a different physiological effect.

Considered from this point of view, the basic anaesthetics which (as shown by the greater slowness with which their effect disappears) form more stable compounds will be more specific in their action, while the indifferent anaesthetics or, as they might perhaps better be called, *general* anaesthetics such as ether and chloroform, which form much more loose combinations, probably of a physical character with the proteid of the protoplasm, will be adapted for more general combination, and will affect all cells more equally, although even here, as has already been pointed out, there is selective action in lessened degree.

We may now pass from the history of the subject to a description of our own experiments, which have chiefly been made with chloroform, although certain experiments have been added with other anaesthetics, which show that the conclusions deduced from the experiments with chloroform hold also for other anaesthetics, and at the outset we desire to acknowledge that our attention was first attracted to the subject by witnessing the experiments of SHERRINGTON and SOWTON[1] upon the effects of chloroform on the excised mammalian heart, fed by a current of RINGER's or LOCKE's solution, and through which, later, a similar current, but containing in addition small amounts of chloroform, could be perfused.

These authors observed that a concentration of chloroform in the LOCKE solution, amounting to only 1 in 100,000, produced a marked and unfailing action in diminishing the extent of the cardiac contractions, and, further, that this effect appeared rapidly after the dilute chloroform solution reached the heart, lasted just as long as the chloroform in this excessively low but yet adequate concentration was passed through, and ceased almost immediately as soon as the normal LOCKE's solution was recurred to, the heart attaining again its normal force.

This effect could be repeated as often as was desired, and there was no cumulative action whatever, that is, no matter how prolonged the passage of the chloroform solution on passing back to the normal LOCKE's solution, the chloroform effect rapily disappeared, and again recovered when the chloroform was once more turned on.

It was this latter effect which suggested the experiments on the chemistry of anaesthesia recorded in this paper. It was quite obvious that the effect of the chloroform upon the cardiac muscle fibres depended solely upon the concentration (solution tension or osmotic pressure) of the chloroform in the cell for the time being, and not at all upon the total amount of chloroform which had been fed to the heart up to the moment of observation.

This experimental fact suggested the view that the effect upon the cell was due to some combination being formed between the protoplasm and the chloroform, and

1. *Thompson Yates and Johnston Laboratories Report*, Vol. 5, Part I, p. 69.

further, that this combination was not a stable fixed one, leading to permanent removal of the protoplasmic activity, but an unstable one, which existed only so long as the pressure of the anaesthetic was kept up to a definite level, and gradually dissociated as the level of chloroform pressure was allowed to fall, and as a result left the protoplasm free for a renewal of its metabolic processes—to choose a familiar analogy, that the protoplasm of a cell undergoing anaesthetization entered into a combination with the anaesthetic, similar to that between haemoglobin and oxygen, unstable in character, and only lasting so long as the pressure of the anaesthetic was kept up.

It occurred to us, as protoplasm is built up chemically of proteid, that a certain amount of evidence as to the formation of such an unstable compound might be obtained, in the first instance, by experimenting with proteids.

We accordingly experimented with the proteids of the blood, and have obtained a number of results which together point to the formation of such compounds as are indicated above.

Our experiments may be described under the following headings :—

1. On the obvious physical and chemical changes produced in serum and in haemoglobin solution by the addition of chloroform.

2. On the relative solubility of chloroform in water, normal saline solution, serum, and haemoglobin solution.

3. On the relative vapour pressures of chloroform when dissolved in water, saline, serum, and haemoglobin solutions respectively, and on the variations in the coefficient of distribution in these solutions.

4. On the solubility of gases in serum and haemoglobin solution in presence of chloroform.

5. On the solubilities of other anaesthetics in water and in serum respectively.

I.—Effects of Chloroform on Serum and on Haemoglobin Solution

On adding chloroform[1] to either serum or haemoglobin solution and allowing the mixture to stand, changes occur which are obvious to the eye, and were to us previously unknown, but on consulting the literature we found that they had been observed by E. Salkowski[2] in using chloroform as a preservative for these fluids, and were also described by Formanek,[3] the bearing of such phenomena upon the question of anaesthesia was not, however, appreciated by these previous observers, who had approached the matter from a different standpoint, and as we have in some respects amplified their observations, and in others have obtained results not quite in accord with theirs, we feel justified in here recording our experiments.

1. The chloroform used for all the experiments described in this communication was presented to us by Messrs. Duncan and Flockhart, of Edinburgh.

2. *Deutsche Med. Wochensch.*, 1888, No. 16; *Zeitsch. f. physiol. Chem.*, Bd. XXXI, 1900, S. 329. The fact that the red corpuscles combine with chloroform is also mentioned by Schmiedeberg, *Arch. f. Heilkunde*, 1867, S. 273.

3. *Zeitsch. f. physiol. Chem.*, Bd. XXIX (1900), S. 416.

It was observed by E. SALKOWSKI in 1888 that blood could not be preserved by adding chloroform, because it gradually became converted into a thick mass.

In 1891 it was observed by HORBACZEWSKI[1] that haemoglobin was precipitated from a solution containing it, and kept at a temperature of 40-50° C., to which chloroform was added as a preservative.

The subject was investigated more minutely by FORMANEK[2] in 1900, and this observer found that a solution of haemoglobin kept at 50-55° C. for some time with chloroform was completely precipitated, the filtrate being entirely free from haemoglobin.

FORMANEK dried and analysed the precipitate, and from the absence of chlorine after fusion with sodium carbonate and potassium nitrate came to the conclusion that the precipitate is not a chloroform compound of haemoglobin. In our opinion this is not a valid proof, as the chloroform need not be so stably combined with the chloroform as to stand drying at 130° C., and subsequent fusion as employed by FORMANEK. The precipitate was dissolved by FORMANEK after thorough washing to remove the chloroform by the addition of a few drops of sodium carbonate solution, and the solution gave the bands of oxy-haemoglobin, and on treatment with ammonium sulphide reduced haemoglobin. FORMANEK also found that blood serum and egg-albumin were precipitated (when the reaction of the fluid was acid or neutral) on keeping at a temperature of 50-55° C. in presence of chloroform. From these experiments this observer came to the conclusion that the precipitate with which he was dealing was a mixture of haemoglobin and other proteids thrown out of solution by the chloroform. It is also stated in this paper that oxy-haemoglobin is only slowly and incompletely precipitated by the action of chloroform at room temperatures.

E. SALKOWSKI, in his later paper,[3] states that blood kept at a temperature of 40° C. for 24-48 hours in presence of chloroform changes to a thick mass, but found that the precipitation of the haemoglobin was not complete under such circumstances.

Regarding the action of chloroform on serum, he states that serum can be preserved in contact with chloroform for years without precipitation at room temperatures, and finds this in agreement with FORMANEK's results, who found, in presence of an alkaline reaction, no precipitation of serum by chloroform even at a temperature of 50-55° C. FORMANEK does not state whether his alkaline reaction is the natural alkaline reaction of the serum. SALKOWSKI also found a precipitating action of chloroform upon solutions of albumose and casein.[4]

Our experiments were conducted with haemoglobin and serum obtained from

1. Quoted by Formánek, *loc. cit.*
2. *Zeitsch. f. physiol. Chem.*, Bd. XXIX (1900), S. 416
3. *Zeitsch. f. physiol. Chem.*, Bd. XXXI (1900), S. 329.
4. The chemical properties of the precipitate caused by the chloroform have been investigated by E. S. Edie, B.Sc., and the results obtained are recorded in the paper immediately following this, p. 195.

pig's blood. The serum used was obtained from clotted blood, and was thoroughly centrifugalized before use. The haemoglobin was in all cases obtained by centrifugalizing the blood corpuscles three times with normal saline, and then laking with distilled water and making up to the same volume as that of the blood taken.

In the case of serum, we found that the fluid acquired, with less than one per cent. of chloroform (and greater quantities up to saturation), a peculiar opalescent and fluorescent appearance, but remained quite transparent to transmitted light. On the addition of over two per cent. of chloroform, there is a tendency to precipitation even in the cold, and at the end of twenty-four to forty-eight hours there is a slight precipitate present, but the effect is much hastened on placing the mixture in an incubator at 40° C., so that it becomes impossible to determine the maximum solubility of chloroform in serum at body temperature. Both in obtaining precipitation in the cold and more rapidly at 40° C. in presence of the natural alkaline reaction of the fluid, our results are at variance with those of FORMANEK and SALKOWSKI. The results were obtained several times in succession.

The marked opalescence in the serum was obtained in preparation of solutions of known concentration in chloroform for purposes of measurement of their vapour pressures, and led us to doubt at first whether we were not dealing with a fine emulsion of chloroform in the serum. Since this point was of vital importance to our experiments on vapour-pressure, we investigated it as completely as possible.

In the first place, examination with the microscope of the opalescent fluid showed no visible globules of chloroform, even with the highest powers.

To make certain of the matter, a current of air from an aspirator was bubbled first through chloroform contained in a WOULFF's bottle, afterwards through a similar bottle containing water, and then, *at the same temperature*, was sent through a third WOULFF's bottle containing serum. By this procedure the serum never came in contact with fluid chloroform, nor with air more highly charged with chloroform vapour than corresponded to the saturation of the air in contact with it or passed through it.

There could, hence, be no condensation of chloroform, and no means by which an emulsion of chloroform, finer even than could be seen with a microscope, could be formed.

Very soon, however, after the chloroform vapour began to pass through, a distinct difference in appearance was observable between the serum and a control placed alongside, and after a time the serum charged with chloroform in this manner was as opalescent as the specimens made in the usual way by shaking with weighed quantities of chloroform, and gave similar results with regard to vapour pressure.

These results show that the marked opalescence is not due to an emulsion of chloroform, and, further, that it is not due to precipitation of proteid in the ordinary sense of the word, for no precipitate can be seen with the microscope in the opalescent fluid.

Further, experiments on the vapour pressure show that the value of this is a long way from the maximum value, which it must obviously possess if an emulsion is present.

A similar opalescence is obtained on adding to serum other organic liquids which possess anaesthetic properties, thus we have obtained it on saturation of serum with ether[1], benzol, xylol, amyl acetate, amyl alcohol, and ethyl acetate, but have not followed the matter up as we have done with chloroform.[2]

In the case of haemoglobin solutions, we have not been able to observe an opalescence similar to that seen in the case of serum, up to the strength at which precipitation begins. In order to study where precipitation commences it is necessary to keep the haemoglobin and chloroform constantly stirred, or otherwise before the chloroform has dissolved the lower layer of haemoglobin solution in contact with the liquid chloroform becomes precipitated. When precautions are taken to prevent this occurring, precipitation is found to take place when about two per cent. of chloroform has been added, that is long before saturation is reached (*vide infra*). This precipitation prevents both the determination of the solubility of chloroform in haemoglobin solutions, and the observation of the vapour pressure with high concentrations. With concentrations of one per cent. or under no precipitation whatever was found to occur. Contrary to SALKOWSKI and FORMANEK, we found that no raising of the temperature was required to cause precipitation of the haemoglobin ; in fact, with strong solutions, the precipitation occurs within a few hours, even in the ice chamber. With time the precipitation is complete, and the precipitate is insoluble in water or saline, but in dilute sodium carbonate it is easily soluble, and we have found that the solution then shows the spectrum of alkaline haematin and not that of oxy-haemoglobin as was found by FORMANEK.

II.—ON THE RELATIVE SOLUBILITY OF CHLOROFORM IN WATER, SALINE, SERUM, AND HAEMOGLOBIN SOLUTION

In so far as we have been able to discover, no attention has been paid by previous experimenters to the maximum amount of chloroform capable of solution in the blood or serum as compared with that taken up by water, or saline solution isotonic with blood.

When any reference is made to the matter, it has been usually assumed on general principles that the serum or plasma will behave like a saline solution of equal concentration and dissolve somewhat less chloroform than water[3]. In other words, that there is no specific action of the proteids or other substances in the plasma.

This supposition we have found experimentally to be entirely erroneous, for both serum and solutions of haemoglobin dissolve much more chloroform than water

1. The opalescence with ether is not nearly so well marked, but increases on standing, and the solution in time becomes extremely viscid.

2. See pp. 186-188

3. See, for example, Overton, *Studien über die Narkose*, S. 98.

or normal saline solution. This fact is of importance in regard to the mode of action, as it definitely points to an interaction between the chloroform and the proteid present.

The presence of fats would, of course, increase the apparent solubility of chloroform in serum, and hence it is necessary in all cases to use perfectly clear serum, free from suspended fat ; this precaution we have always been careful to observe ; and, in addition, the serum has always been centrifugalized.

In this connexion, it may be added that the haemoglobin solutions which we have employed could not contain any appreciable amount of fatty matter, and hence the high solubilities which we have observed could only arise from chemical interaction between the haemoglobin and the chloroform.

Methods for Determining Maximum Solubility

Three methods have been used in the determination of the maximum solubility of chloroform in the solvents mentioned above, which have given concordant results, and shown that the solubility in proteid solution is much higher than in water or saline.

In the first method we have determined the amount of chloroform dissolved by obtaining the product of volume and vapour pressure at low pressure and with a small volume of fluid, so that practically all the chloroform was simply pumped off into the vacuum. In this method the volume of fluid experimented with is necessarily small, and this gives rise to experimental error of measurement, which is added to by the volume measured being large and pressure small, so that the results are only approximative, yet it is observable that they confirm those obtained by the more accurate methods described below.

The details of the method are described in the succeeding section on the relationship between vapour pressure and concentration of chloroform, and it need only be mentioned here that we have obtained solubilities of 0·95 per cent. in normal saline (0·75 per cent.), 3·33 per cent. in serum, and 4·42 per cent. in whipped blood by this method.

The second method employed consists in weighing out known amounts of chloroform into water and serum and haemoglobin solution respectively, and then determining by direct observation that concentration in each case at which the chloroform ceased to be dissolved.

This method of observation is made easy in the case of chloroform by the high specific gravity of that fluid, as a result of which, on inverting the flask in which the determination is being made, even minute globules of undissolved chloroform can be seen falling through the fluid.

The determinations of solubility by this method are made on the following plan : Pure chloroform is dropped from a fine capillary pipette into a tared graduated flask

of 25, 50, or 100 c.c., and carefully weighed to definite amount, corresponding, when the flask has been filled by the solvent under experiment, to a definite percentage of chloroform. A series of such flasks is prepared, and immediately after each flask is filled with the desired solvent and either shaken thoroughly by hand until solution is complete, or placed on a rotary shaking machine. After the lapse of several days, during which time the flasks are never opened and are kept shaken up, it is noticed at what level of concentration the chloroform ceases to be completely dissolved, and so the solubility is determined.

The lower strengths of known value short of saturation and also the saturated solutions so prepared were kept and used also for the experiments on vapour pressure at varying concentration described in the next section of this paper.

The following results were obtained by the application of this method at room temperatures, approximately (13° C.), for the percentage by weight dissolved :—

Water, 0·8 per cent. dissolved, 0·9 per cent. dissolved, 1 per cent. not dissolved completely. Estimated solubility, 0·95 per cent.

Saline Solution (0·75 per cent. sodium chloride in water), 0·7 per cent. dissolved, 0·8 per cent. dissolved, 0·9 per cent. not dissolved. Estimated solubility about 0·83 per cent.

Serum, 3 per cent. dissolved, 3·5 per cent. dissolved, 4 per cent. all dissolved save a few small globules.

Haemoglobin Solution or Blood.—Over 6 per cent. by weight is taken up when chloroform is shaken with blood or haemoglobin solution of equal strength to the blood, prepared from blood by centrifugalizing several times with saline and subsequent laking with distilled water, and no globules of chloroform can be seen on careful examination with the microscope. But the solution rapidly changes in colour, and a precipitate is thrown out on standing, as above described, which is quite insoluble in water or saline, but easily soluble in dilute sodium carbonate solution, and then gives the spectrum of alkaline haematin.

The blood begins to give this precipitate when about 1·5 per cent. of chloroform has been added at room temperature, but with a lower concentration, and more rapidly when heated to body temperature in the incubator. Two per cent. of chloroform gives a precipitate in the cold, and on heating to 40° C. a red flocculent precipitate leaving a clear colourless fluid above.

The third method for determining the solubility of chloroform in the fluids experimented with consists in shaking up thoroughly for several hours with an excess of chloroform, and then pipetting off and determining the amount of chloroform in the solution.

The difficulty here is a rapid and accurate method of determining the amount of chloroform contained in a measured volume of the given saturated solution.

The procedure finally employed for this purpose was as follows :—

A measured volume (usually 10 c.c.) of the fluid saturated with chloroform is placed in a flask fitted airtight with a double bored cork, and a stream of hydrogen is aspirated through the solution, the oxygen present in the flask and connexions is absorbed by passing through alkaline pyrogallate, and the mixture of hydrogen and chloroform is then burnt by passing over heated palladium asbestos placed in a very short combustion tube. All the chlorine in the chloroform is thus burnt to hydrochloric acid, and the amount of this absorbed in standard alkali is then estimated, first by back titration against standard acid, and then further checked, either by volumetric titration with standard silver nitrate solution or by gravimetric determination as silver chloride.

The serum used in these determinations was examined for chloroform emulsion by the microscope, but no undissolved chloroform in suspension was observed. The precipitate in serum at atmospheric temperature obtained by this method of shaking up with excess of chloroform was very dense, so that the serum became quite opaque.

The results obtained by employing this method were as follows :—

Distilled water, dissolved 0·95 per cent., and serum, dissolved 5·08 per cent.

III.—On the Vapour Pressure of Chloroform Dissolved in Varying Concentration in Water, Saline, Serum, and Haemoglobin Solutions Respectively

A determination of the vapour pressure of an anaesthetic in solution at varying concentrations in serum, in haemoglobin, or in blood, is of high practical importance, since it is upon the relationship of this vapour pressure to the concentration of the solution that the amount of anaesthetic taken up by the blood circulating through the lungs depends.

It has hitherto been taken for granted that the Dalton-Henry law can be applied, and that the amount of anaesthetic taken up is strictly proportional to, and varies directly with, the percentage of the vapour of the anaesthetic in the inspired air.

This has never, however, to our knowledge, been experimentally tested, and it seemed to us desirable to attempt such a determination. We have investigated from this point of view solutions of chloroform in serum, haemoglobin solution (of equal strength in haemoglobin to the blood from which the haemoglobin was prepared) and whipped blood, and have contrasted the pressures obtained with those of solutions in chloroform, in water, and normal saline at equal concentrations.

The vapour pressures have been measured corresponding to concentrations ranging from considerably below the anaesthetizing values for chloroform vapour pressure in air (viz., 8-10 mm.), observed by Paul Bert, up to the saturation points in most cases.

Apparatus

The instrument employed for this purpose was a form of ' differential densimeter,' which, after passing through many modifications, took the form represented in the accompanying sketch (Fig. 1), which is drawn approximately to a scale of one-quarter.

The two tubes shown are exactly similar, and are graduated in cubic centimetres and tenths in the upper portion, and in centimetres in the lower and wider portion.

The tubes are connected as shown by means of thick-walled rubber tubing and a glass Y-piece to a stout glass mercury receiver capable of holding more than enough mercury to fill both tubes and their connexions.

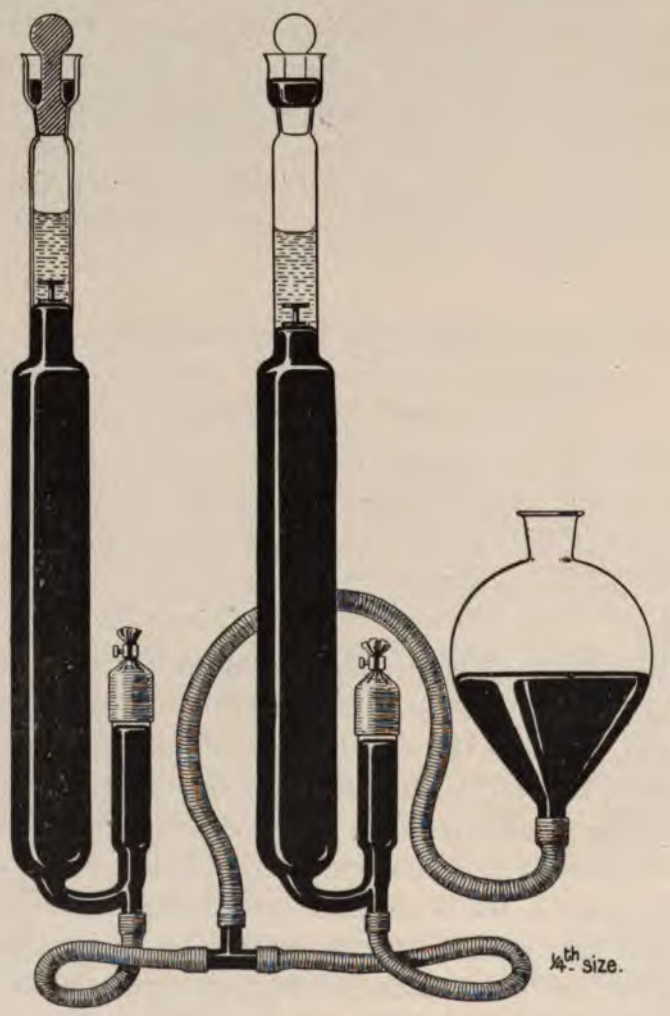


Fig. 1.—Diagram of Differential Densimeter.

The tubes are held in a vertical position by clamps attached to the strong vertical iron bar of a massive retort stand, and each tube is capable of being moved in its clamp vertically up and down for purposes of adjusting the mercury levels.

In order to keep a constant temperature (in the case of the experiments carried out at body temperature) the upper portion of each tube, from about the middle of the wide part to the level of the stopper at the top, was encased in a hot-water jacket of the form shown in detail in Fig. 2, and omitted for clearness from Fig. 1.

It was found convenient to use for the outer glass tubing of this jacket the largest size of a common variety of paraffin-lamp chimney, which measured 8 cm. in diameter above, bulged out as shown to 9 cm., and then narrowed below to 6 cm.

The wider top and bulge were found very useful in facilitating the introduction and vigorous movements of the bar electro-magnet used as a stirrer in connexion with the iron stud (seen in Figs. 1 and 2, at the top of the mercury), which was dropped into each tube, and, during an experiment, agitated up and down so as to thoroughly mix the fluid under experiment, and so bring it into equilibrium more rapidly with the vapour in the space above it.

The hot-water jacket was made watertight below by means of an india-rubber cork, as shown (Fig. 2); the rubber cork was also bored in each case for two narrow glass tubes, for the purpose of carrying water to and from the jacket. The two tubes carrying the water in stopped about 2 cm. above the upper surface of the rubber cork in each tube, so as to prevent blocking by mercury accidentally run over from the top when the inner tubes were being cleared out at the termination of a measurement. The outer ends of these two tubes were attached by means of narrow rubber tubings and a glass Y-tube to a bath of hot water, placed at a higher level, and a screw-down clip on each rubber tube regulated the flow until a thermometer placed in the corresponding hot-water jacket showed the desired temperature. A constant level of water was kept up automatically in the warm supply bath, and its temperature was regulated so as to lie 2-3° above that of the jackets. The two outflow tubes passed up, as shown, inside the jacket to the level of the ground in glass stopper, and their outside ends were connected by means of rubber tubing to the waste pipe.

In Fig. 1 the upper portion of the left-hand tube is shown in section, and that of the right-hand one in outline. The ground in stoppers shown were found, when sealed with mercury, to be much more effectual against minute leakages, which entirely vitiate the results, than any form of tap, and they are also much more convenient for introducing the solutions to be experimented upon, and for cleaning out the apparatus. Further, since they do not require to be operated between the commencement and termination of each determination of vapour pressure, they are better adapted to their particular purpose than a tap. In the course of our experiments, we also found it necessary to be able to dilute a solution with more of its solvent without allowing it to come in contact with any appreciable volume of air, and for this purpose found the stopper arrangement most convenient.

The side tube shown at the lower end of each main tube was designed to trap air which was found to slowly leak in through the rubber pressure tubing, when a

vacuum was established by lowering the mercury receiver, and for a long time was a source of annoyance. We subsequently learned that the device had been first introduced by Lord RAYLEIGH. At the end of an experiment, any air which has collected is discharged by raising the mercury-holder, opening the screw-clip shown, and allowing enough mercury also to pass through to form a seal in the rubber tubing above the clip.

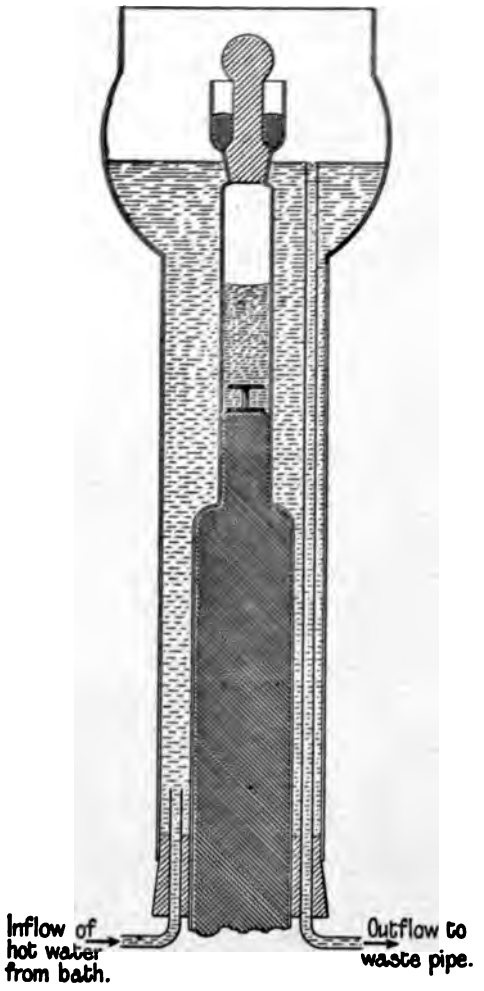


FIG. 2.—Section taken through the upper portion of one tube of the Differential Densimeter and the hot-water jacket, showing the inflow and outflow tubes. Scale, one-third.

The mercury-holder was suspended by means of a ring and loop of wire attached around the bulb from a vertical rod, swivel, and hook, possessing a slow screw movement in a block attached to a horizontal rod fixed in a clamp, which could be moved up and down on a heavy retort stand. For large movements, the clamp was slid up or down the retort standard, and for fine movements the screw was raised or lowered in its block.

In using the apparatus, the two vertical tubes are first placed at the same level, the mercury-holder is filled with mercury, and, with the two glass stoppers out, the whole apparatus is filled with mercury, the two stoppers are next inserted, enough mercury being left above them to form a seal, and the mercury-holder is then lowered until the two vertical tubes become evacuated. The receiver is then raised again to the level of the stoppers, and any bubble of air found is discharged.

The apparatus is now ready for an experiment, and, with stoppers out, the levels of mercury are adjusted until there is an equal volume left above the mercury on each side. A given volume of the solvent (say 5 c.c.) is now introduced on the one side (say left), and an equal volume of the solution of chloroform in the same solvent on the other side. In each case, immediately after the fluid has been introduced, the stopper is inserted, care being taken to prevent any air being included, either as a bubble at the mercury surface, or between the surface of the introduced fluid and the stopper. To achieve the latter end, we have almost always introduced above the mercury 2 or 3 c.c. more than the required quantity, so that it stood in the neck and slightly above, and then, by easing the stopper and gently adjusting the level of the mercury-holder, have brought the level of the mercury in the tube to the desired volume mark. After the solvent on the one side and the solution of chloroform of the desired strength on the other side have been successfully introduced in equal volume, and without any bubble of air, the mercury-holder is lowered until a space containing vapour has appeared on each side. The level of the mercury will be found to be lower on the chloroform side, and it is obvious, the instrument being independent of variations in atmospheric pressure, and the only different factor being the added chloroform on the one side,[1] that the difference in pressure will give the vapour pressure directly for that strength of chloroform solution at that particular temperature.

There is hence no need to determine pressure due to dissolved gases on the two sides,[2] or pressure of aqueous vapour, since these balance, and the quickness with which readings can be directly obtained makes it possible to carry out a long series of determinations at varying strengths, without the proteid solutions having time to undergo bacterial change.

Certain precautions have to be taken, however, and corrections made which may here be mentioned :—

1. Before taking a reading it is essential to move the tubes vertically and adjust the levels until the volumes of the vapour spaces above the upper aqueous solution meniscus in each case are exactly equal, otherwise inequality in pressure of gases pumped off on the two sides gives rise to an error, which is greater the smaller the vapour space.

1. There will be a small difference in the pressure of water vapour on the two sides, due to there being a stronger solution on the chloroform side, but this is in all cases too minute compared to the pressure of the chloroform vapour to make any appreciable error.

2. Slight differences in dissolved gases gave a disturbance with very dilute chloroform solutions, and this was later obviated by pumping the gases off (*vide infra*).

2. Before taking a reading, there must be certainty that each fluid is in equilibrium with its vapour space. This is shown by absence of variation when the apparatus is left at rest.

For rapid and accurate working the mechanical stirring by means of the studs and magnet is indispensable, for even after the lapse of an hour when at rest the solution has not completely discharged its proper amount of chloroform into the vapour space. When once the control, containing, of course, no chloroform, has been thoroughly stirred it remains constant, and need not be changed at the end of each determination, but can be used throughout an entire experiment.

By vigorous stirring, equilibrium can be attained in five to ten minutes, and the level does not afterwards change, no matter how long stirring, and observation be kept up. This important experimental observation we have taken occasion to verify several times during our experiments.

3. For very accurate working, especially with the dilute solutions and low pressures, it is necessary in the case of serum and haemoglobin to pump off the dissolved gases by means of a Töpler pump, otherwise these come off unequally from solvent and solution and disturb the results at the low pressures. The chloroform solutions are then made up from the pumped-out solvent, which also must be used for control and for making the dilutions.

4. The temperature must be the same in the jackets surrounding each tube at the time when each reading is taken, and in a series of determinations at varying strength and a constant temperature, that temperature must be closely maintained throughout. The temperature error is a maximum when the solutions are near saturation, for then the variation in vapour pressure per degree is very large; fortunately here the differences in level under observation are also very large, which diminishes the percentage error arising from small deviations in temperature.

At concentrations away from saturation, the variations arising from small differences in temperature approximately obey the gas law, and under the conditions of our experiments become quite negligible.

5. A correction must be made in all cases upon the concentration of the solution introduced into the tube for the amount of chloroform pumped off from the solution into the vapour space. This correction is, of course, larger in the case of the more concentrated solutions with high vapour pressures.

This has been done in the experiments of which records are given below, and accounts for the concentrations not being exact percentages or small fractions of exact percentages.

The amount of chloroform in the vapour space is readily calculated from the product of the observed vapour pressure and the volume of the vapour space, and this amount deducted from the quantity contained in the chloroform solution when it was introduced, gives the necessary datum for calculating the concentration in chloroform of the solution corresponding to the observed vapour pressure in the vapour space.

The ratio of the vapour concentration in the fluid to the concentration in the vapour space gives the coefficient of distribution (*coefficient de partage*, *Theilungs-coefficient*) ; this should remain constant if the absorption of the chloroform vapour by the liquid were normal and strictly proportional to the vapour pressure, and if it varies it points to a physical or chemical aggregation or compound between the chloroform and the fluid or its constituents (*vide infra*).

Method of Reading.—The readings were taken with a cathetometer,[1] placed about four feet from the tubes, both for greater accuracy in reading than direct measurement would give and to avoid changes in temperature.

Two Methods of Experimentation.—We have employed two different methods of experimentation in investigating the variation in vapour pressure with varying concentration. It is obvious that the concentration of a measured volume of a strong solution introduced into the densimeter may be diminished by pumping off more and more chloroform, by increasing the volume of the vapour space above the solution.

A series of readings of differences in pressure may thus be obtained in which the vapour space on the two sides is kept equal and of known and increasing value throughout the series. This method we have called the method of *variable vapour space.*

On the other hand, a series of solutions of known and steadily diminishing or increasing concentration may be introduced into the densimeter and measured one after another as to their vapour pressure, in each case with a known fixed volume of vapour space. As stated above, the concentration at which each vapour pressure in the series is measured is then accurately known. This method we have called the method of *constant vapour space.*

Method of Variable Vapour Space.—In this method, unless the volume of the tubes of the densimeter is very large, the volume of solution and solvent respectively introduced must be very small. We have usually taken $\frac{1}{2}$ c.c. on each side, either of a saturated solution or of a very strong solution of known strength, and, by altering the levels of the tubes and mercury receiver, have taken a long series of readings, in each case with equal volume of vapour space on each side, at every increasing volume of vapour space, until the difference in pressure became of small value, and the product of volume and vapour pressure became approximately constant, showing that practically all the chloroform had been pumped off from the solution. This constant product then gave the necessary datum for calculating the concentration of the original solution introduced into the densimeter, the product of the vapour pressure and volume at each stage gave the datum for calculating the quantity of chloroform pumped off from the solution, and, therefore, for deducing the corresponding

1. The instrument used was made by Pye and Co., of Cambridge, and, by means of a vernier and divided screw-head, read to $\frac{1}{100}$ mm. We have frequently observed that we were able to take readings within five divisions, that is $\frac{1}{20}$ mm., which is far within the accuracy of other portions of our determinations.

concentration of solution. Or, also, by plotting vapour pressures as abscissae, and the product of vapour pressure and volume of vapour space as ordinates, the ratio of vapour pressure and amount of chloroform absorbed at each stage could be shown.

The method of *variable vapour space* has, however, two working disadvantages which caused us in the end to abandon it and replace it by the method of *constant vapour space.* The first objection is that the amount of solution taken is small, hence it is difficult to measure it with accuracy, and to make it equal on the two sides.

The second objection is that at the low concentrations the increase of volume for a small fall in pressure is very large, and hence the determinations become inaccurate, a small error in pressure-reading making a large deviation. The values for high pressures are also inaccurate., but for a different reason ; these readings are taken with small volumes of vapour space, and unless the vapour spaces are accurately equal on the two sides, there is a large disturbance due to inequality in pressure of the previously dissolved gases pumped off on the two sides.

The results, however, in the intermediate pressures are accurate and are given below, as they confirm those given by the other method.

Method of Constant Vapour Space.—In using this method we have always introduced a volume of 5 c.c. of the solvent on one side, and 5 c.c. of a solution of known strength on the other, and have invariably adjusted the levels so that at the temperatures of observation there was a vapour space of exactly 5 c.c. on each side.

In some cases we have started with a saturated solution of chloroform, and have then made dilutions of different percentages of that solution in the manner described below. In the later experiments we found it, however, more expedient, on account of knowing the exact concentration directly, to prepare a solution of known strength, say one per cent., and for the more dilute solutions to use various percentage dilutions of this stock solution. The more concentrated solutions were obtained by making up solutions varying by one per cent. in strength, and one-half per cent. differences were got by mixing these with each other in equal proportions. For percentages less than 0·1 per cent., a 0·1 per cent. solution was first prepared by making a ten-fold dilution of the one per cent. solution, and this 0·1 per cent. solution was then diluted similarly to the one per cent. solution.

Precautions in Preparing, Preserving, in Unaltered Strength, and Diluting by Known Amounts, of the Solutions Used

In working with the solutions it is indispensable that due precautions be taken against loss by escape of chloroform into the air during the various manipulations.

If a portion, for example, of a stock solution of known strength be pipetted off for use in the densimeter, and then the stock bottle be merely stoppered, the air space over the portion of solution left in the bottle rapidly becomes charged with chloroform vapour at the expense of the stock solution, and a second sample taken

later from the same bottle will be found to be weak and give a wrong result. The way to guard against this is to fill the bottle with mercury up to the neck immediately after drawing off, and at once stopper up. Similar devices were employed in all dilutions to complete the process out of contact with air.

The stock solutions were made by direct weighing, by dropping from a fine pipette into graduated glass-stoppered flasks of 25, 50, or 100 c.c. capacity, according to the amount required, immediately filling up to the mark with the particular solvent, and setting at once upon a slowly rotating disc, driven at such a rate that the chloroform globules have just time to fall through the solution each time the flask is inverted. In this way a rapid solution is effected, so saving much time. Further, in certain cases such shaking is indispensable, for a solution of haemoglobin left in contact with even a small amount of chloroform without continuous shaking soon forms a precipitated layer at the bottom.

The stock solutions were kept carefully stoppered, unless at the moment of introducing the pipette to remove a portion for experiment, and then the space within the bottle was always filled to the stopper with mercury in the manner above described.

We found much difficulty owing to leakage of chloroform during dilution in the case of the strengths intermediate between the stock strengths, until it occurred to us to make these dilutions in the densimeter itself. For this purpose we always placed the mercury level at 10 c.c. below the stopper on the side of the densimeter in which the mixing was to be carried out, then the proper amounts of the two solutions necessary to give the desired strength was drawn up in two graduated pipettes and run into the densimeter tube, the stopper was then inserted, including only a minute bubble of air, and now by thorough agitation of the iron stud through the fluid by means of the electro-magnet a thorough mixing of the two fluids was attained, then by raising the mercury-holder and slightly easing the stopper 5 c.c. were allowed to escape, the mercury level being placed accurately at 5 c.c. A seal of mercury was finally dropped in above the stopper, and so the dilution was effected.

For example, to obtain a dilution of 1·5 per cent., 5 c.c. of one per cent. solution and 5 c.c. of two per cent. solution were drawn off into pipettes, placed in the densimeter and mixed as above described ; to obtain a 0·4 per cent. solution, 6 c.c. of the solvent were taken and 4 c.c. of a one per cent. solution, and similarly treated ; to obtain a 0·03 per cent. solution, 7 c.c. of solvent and 3 c.c. of 0·1 per cent. solution were taken, and so on.

Certain of our experiments were carried out at room temperature and others approximately at body temperature (40° C.) ; the following protocols and accompanying curves show some of the typical results obtained, which have been confirmed in most cases by duplicates :—

Variable Vapour Space

Experiment I.—Distilled water containing approximately 0·78 per cent. of

chloroform. Half-a-cubic centimetre was introduced into each tube of the densimeter of the chloroform water on the one side and of the same distilled water without chloroform on the other. The temperature at which the experiment was carried out was 17° C., and the volume at which readings of pressure were taken varied from 2 to 50 c.c. The percentage of chloroform in the water was not known directly, but was calculated by extrapolation of the curve showing V.P., see Curve I, Fig. 3.

The following table gives the results of the experiment, which are also shown graphically in Curve I, Fig. 3, in which the abscissae show pressures of chloroform vapour and the ordinates the product of the volume and pressure of vapour. In Curve I, Fig. 4, the same experiment is shown with pressures of chloroform vapour as abscissae, and percentage of chloroform dissolved at each pressure as ordinates :—

EXPERIMENT I.—DISTILLED WATER

TEMPERATURE 17° C.

Volume of vapour space	Pressure of chloroform in vapour space in mm. of mercury	Percentage by weight of chloroform in vapour space	Weight in grammes of chloroform in vapour space	Weight in grammes of chloroform in solvent	Percentage by weight of chloroform in solvent	Coefficient of distribution between vapour space and solvent
2	74·61	0·04957	0·00099	0·00293	0·586	1 : 11·8
3	60·62	0·04027	0·00121	0·c0271	0·542	1 : 13·4
5	51·29	0·03408	0·00170	0·00222	0·444	1 : 13·0
6	47·69	0·03168	0·00190	0·00202	0·404	1 : 12·7
7	43·08	0·02862	0·00197	0·00195	0·390	1 : 13·6
8	38·91	0·02585	0·00207	0·00185	0·370	1 : 14·3
9	35·98	0·02390	0·00215	0·00177	0·354	1 : 14·8
10	34·36	0·02283	0·00228	0·00164	0·328	1 : 14·4
12	28·83	0·01915	0·00230	0·00162	0·324	1 : 16·9
15	26·16	0·01738	0·00263	0·00129	0·258	1 : 14·8
20	22·62	0·01503	0·00301	0·00091	0·182	1 : 12·1
25	17·65	0·01173	0·00293	0·00099	0·198	1 : 16·9
30	15·84	0·01052	0·00316	0·00076	0·152	1 : 14·5
35	14·37	0·00954	0·00334	0·00058	0·116	1 : 12·2
40	12·43	0·00835	0·00334	0·00058	0·116	1 : 13·9
45	11·87	0·00787	0·00354	0·00038	0·076	1 : 9·7
50	10·54	0·00700	0·00350	0·00042	0·084	1 : 12·0

Experiment 2.—Serum containing approximately 1·65 per cent. of chloroform, the amount being determined by extrapolation of Curve 2, Fig. 3. Experiment conducted in all respects similarly to Experiment 1. Graphic records of results shown by Curve 2, in Figs. 3 and 4.

EXPERIMENT II.—SERUM

TEMPERATURE 18° C.

Volume of vapour space	Pressure of chloroform in vapour space in mm. of mercury	Percentage by weight of chloroform in vapour space	Weight in grammes of chloroform in vapour space	Weight in grammes of chloroform in solvent	Percentage by weight of chloroform in solvent	Coefficient of distribution between vapour space and solvent
1	115·63	0·07655	0·00077	0·00746	1·492	1 : 19·5
1·5	1 : 1·85	0·07405	0·00111	0·00712	1·424	1 : 19·2
2	104·27	0·06904	0·00138	0·00685	1·370	1 : 19·9
3	98·15	0·06498	0·00195	0·00628	1·256	1 : 19·3
4	89·89	0·05763	0·00237	0·00586	1·172	1 : 20·4
5	87·05	0·05277	0·00264	0·00559	1·118	1 : 21·2
6	79·70	0·04975	0·00299	0·00524	1·048	1 : 21·7
7	75·14	0·04730	0·00331	0·00492	0·984	1 : 20·6
8	71·46	0·04487	0·00359	0·00464	0·928	1 : 20·7
9	67·16	0·04447	0·00402	0·00421	0·842	1 : 18·9
10	63·24	0·04187	0·00419	0·00404	0·808	1 : 19·3
12	58·05	0·03843	0·00461	0·00362	0·724	1 : 18·9
15	52·31	0·03463	0·00519	0·00304	0·608	1 : 17·6
20	43·64	0·02889	0·00577	0·00246	0·492	1 : 17·0
25	34·97	0·02315	0·00579	0·00244	0·488	1 : 21·1
30	32·38	0·02144	0·00643	0·00180	0·360	1 : 12·1
35	28·20	0·01867	0·00653	0·00170	0·340	1 : 18·2
40	25·44	0·01684	0·00674	0·00149	0·298	1 : 17·7
45	22·40	0·01483	0·00668	0·00155	0·310	1 : 20·9
50	21·03	0·01392	0·00696	0·00127	0·254	1 : 18·3
60	17·91	0 01186	0·00712	0·00111	0·222	1 : 19·0
70	16·03	0·01061	0·00743	0·00080	0·160	1 : 15·1
80	13·93	0·0092	0·00737	0·00086	0·172	1 : 18·7
90	12·12	0·c080	0·00722	0·00101	0·202	1 : 24·3
100	10·70	0·0071	0·00708	0·00115	0·230	1 : 32·4
125	8·77	0·0058	0·00726	0·00097	0·194	1 : 33·4
150	7 91	0·0052	0·00786	0·00037	0·074	1 : 14·3

FIG. 3

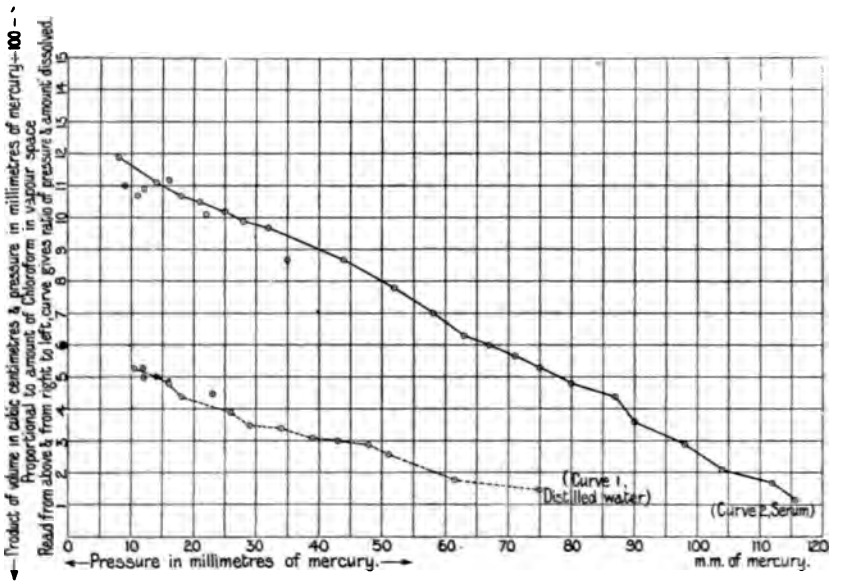
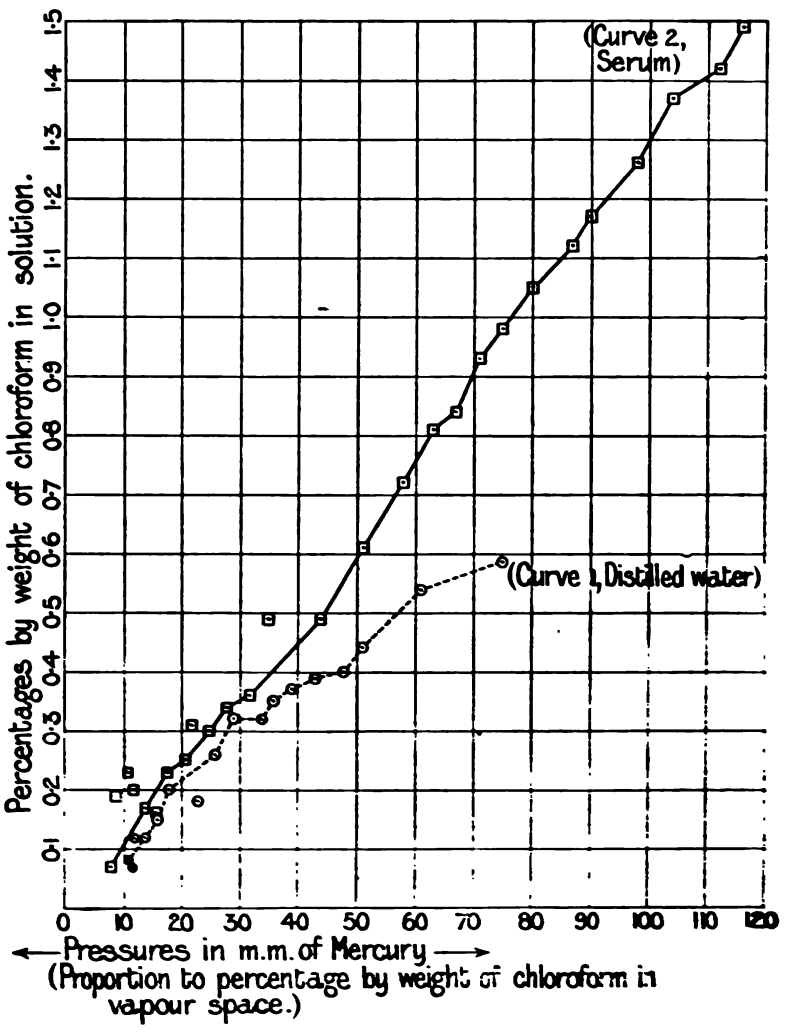

Product of volume in cubic centimetres & pressure in millimetres of mercury ÷ 100 —
Proportional to amount of Chloroform in vapour space.
Read from above & from right to left, curve gives ratio of pressure & amount dissolved.
(Curve 1, Distilled water)
(Curve 2, Serum)
0 10 20 30 40 50 60 70 80 90 100 110 120
←Pressure in millimetres of mercury.→
m.m. of mercury.

FIG. 4


(Curve 2, Serum)
(Curve 1, Distilled water)
Percentages by weight of chloroform in solution.
0·1 0·2 0·3 0·4 0·5 0·6 0·7 0·8 0·9 1·0 1·1 1·2 1·3 1·4 1·5
0 10 20 30 40 50 60 70 80 90 100 110 120
←Pressures in m.m. of Mercury→
(Proportion to percentage by weight of chloroform in vapour space.)

Experiment 3.—Saline solution containing 0·75 per cent. of sodium chloride and, approximately, 0·95 per cent. of chloroform, determinedfrom product of pressure and volume after pumping off. Five c.c. of the saline introduced on one side as a control and 5 c.c. of the chloroform solution on the other, and a vapour space of 5 c.c. being formed on each side. Temperature of experiment, 15° C. The results are shown graphically in Curve 1, Fig. 5.

EXPERIMENT III.—SALINE

TEMPERATURE 15° C.

Percentage of chloroform by weight in solution originally introduced	Pressure of chloroform in vapour space in mm. of mercury	Percentage by weight of chloroform pumped off into vapour space	Percentage of chloroform by weight remaining in solution	Coefficient of distribution between vapour space and solvent
0·0024	0·56	0·0004	0·0020	1 : 5·0
0·0048	1·78	0·0012	0·0036	1 : 3·0
0·0095	2·76	0·0018	0·0077	1 : 4·3
0·0190	4·44	0·0030	0·0160	1 : 5·3
0·0286	5·65	0·0038	0·0248	1 : 6·5
0·0381	6·79	0·0045	0·0336	1 : 7·2
0·0476	7·38	0·0049	0·0427	1 : 8·7
0·0571	8·34	0·0056	0·0515	1 : 9·2
0·0666	10·14	0·0068	0·0598	1 : 8·8
0·0762	11·28	0·0075	0·0687	1 : 9·2
0·0857	12·25	0·0082	0·0775	1 : 9·5
0·0952	14·43	0·0096	0·0856	1 : 8·9
0·1904	25·68	0·0172	0·1732	1 : 10·1
0·2856	39·76	0·0256	0·2600	1 : 10·2
0·3808	47·96	0·0321	0·3487	1 : 10·9
0·4760	60·16	0·0402	0·4358	1 : 10·8
0·5712	72·21	0·0483	0·5299	1 : 10·8
0·6664	79·75	0·0534	0·6130	1 : 11·5
0·7616	88·67	0·0593	0·7023	1 : 11·8
0·8568	99·16	0·0663	0·7905	1 : 11·9
0·9520	110·39	0·0738	0·8782	1 : 11·9

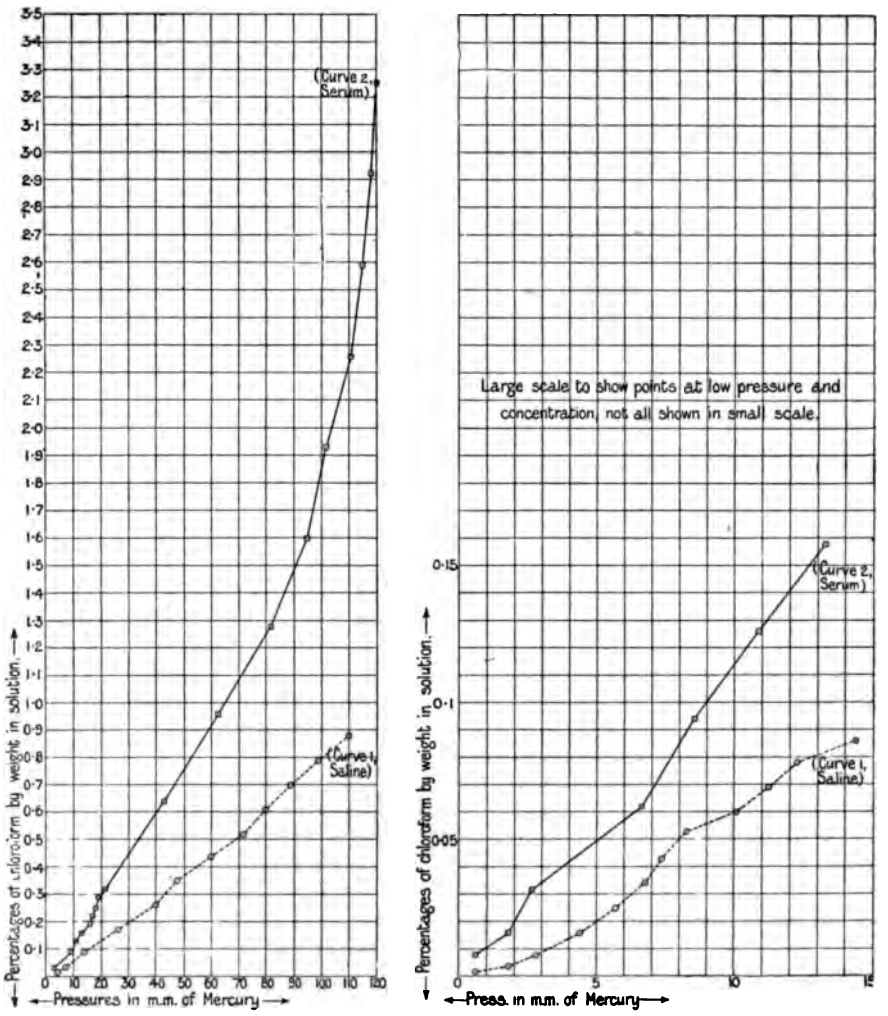
Experiment 4.—Serum containing approximately 3·33 per cent. of chloroform determined as in Experiment 3. Experiment performed similarly to Experiment 3, and temperature also 15° C. Results plotted in Curve 2, Fig. 5.

EXPERIMENT IV.—SERUM

TEMPERATURE 15° C.

Percentage by weight of chloroform originally introduced	Pressure of chloroform in vapour space in mm. of mercury	Percentage by weight of chloroform pumped off into vapour space	Percentage by weight of chloroform remaining in solution	Coefficient of distribution between vapour space and solvent
0·0083	0·66	0·0004	0·0079	1 : 19·7
0·0167	1·82	0·0012	0·0155	1 : 12·8
0·0333	2·74	0·0018	0·0315	1 : 17·5
0·0666	6·66	0·0045	0·0621	1 : 13·8
0·0999	8·59	0·0057	0·0942	1 : 16·5
0·1332	10·85	0·0073	0·1259	1 : 17·3
0·1666	13·28	0·0089	0·1577	1 : 17·7
0·1999	15·54	0·0104	0·1895	1 : 18·2
0·2332	16·96	0·0113	0·2219	1 : 19·5
0·2665	18·37	0·0123	0·2543	1 : 20·7
0·2998	19·19	0·0128	0·2870	1 : 22·4
0·3331	22·49	0·0150	0·3181	1 : 21·2
0·6662	43·02	0·0281	0 6381	1 : 22·7
0·9993	63·15	0·0422	0·9571	1 : 22·7
1·3324	82·16	0·0550	1·2774	1 : 23·5
1·6655	95·30	0·0638	1·6017	1 : 25·1
1·9986	101·64	0·0680	1·9306	1 : 28·4
2·3317	110·53	0·0740	2·2577	1 : 30·3
2·6648	115·30	0·0771	2·5877	1 : 33·6
2·9979	117·53	0·0786	2·9123	1 : 37·1
3·3310	120·41	0·0810	3·2500	1 : 40·1

FIG. 5


(Curve 2, Serum)
(Curve 1, Saline)
Percentages of chloroform by weight in solution.
Pressures in m.m. of Mercury
Large scale to show points at low pressure and concentration, not all shown in small scale.
(Curve 2, Serum)
(Curve 1, Saline)
Percentages of chloroform by weight in solution.
Press. in m.m. of Mercury

Experiment 5.—Saline containing 0·75 per cent. of sodium chloride and the amounts of chloroform shown in the table, the maximum being 0·8 per cent. originally in solution. In this and succeeding experiments the amount of chloroform was known directly by weighing out as described above. The temperature in this and succeeding experiments was 40° C. The results of this experiment are shown in Curve 1, Fig. 6.

EXPERIMENT V.—SALINE (0·75 PER CENT.)

TEMPERATURE 40° C.

Percentage by weight of chloroform originally introduced	Pressure of chloroform in vapour space in mm. of mercury	Percentage by weight of chloroform pumped off into vapour space	Percentage by weight of chloroform remaining in solution	Coefficient of distribution between vapour space and solvent
0·04	11·75	0·0072	0·0328	1 : 4·6
0·06	20·59	0·0127	0·0473	1 : 3·8
0·08	23·29	0·0143	0·0657	1 : 4·6
0·1	29·35	0·0181	0·0819	1 : 4·5
0·2	65·62	0·0404	0·1596	1 : 4·0
0·3	99·83	0·0615	0·2385	1 : 3·9
0·4	129·11	0·0795	0·3205	1 : 4·0
0·5	166·04	0·1022	0·3978	1 : 3·9
0·6	196·05	0·1207	0·4793	1 : 4·0
0·7	224·04	0·1379	0·5621	1 : 4·1
0·8	262·52	0·1616	0·6384	1 : 4·0

Experiment 6.—Serum containing amounts of chloroform shown in table by direct weighing. Results shown in Curve 2, Fig. 6.

EXPERIMENT VI.—SERUM

TEMPERATURE 40° C.

Percentage by weight of chloroform originally introduced	Pressure of chloroform in vapour space in mm. of mercury	Percentage by weight of chloroform pumped off into vapour space	Percentage by weight of chloroform remaining in solution	Coefficient of distribution between vapour space and solvent
0·04	6·80	0 0042	0·0358	1 : 8·5
0·05	8·51	0·0052	0·0448	1 : 8·6
0·06	11·64	0·0072	0·0528	1 : 7·3
0·08	15·09	0·0093	0·0707	1 : 7·6
0·1	19·74	0·0122	0·0878	1 : 7·2
0·15	34·12	0·0210	0·1290	1 : 6·1
0·2	47·27	0·0291	0·1709	1 : 5·8
0·3	77·43	0·0477	0·2523	1 : 5·3
0·4	91·46	0·0563	0·3437	1 : 6·1
0·5	105·31	0·0663	0·4337	1 : 6·5
0·6	149·54	0·0921	0·5079	1 : 5·5
0·8	205·22	c·126	0·674	1 : 5·3
1·0	240·49	0·148	0·852	1 : 5·8
1·5	317·74	0·195	1·305	1 : 6·7
2·0	329·75	0·203	1·797	1 : 8·9
2·5	339·81	0·209	2·291	1 : 10·9
3·0	332·36	0·205	2·795	1 : 13·6
3·5	332·00	0·204	3·296	1 : 16·2
4·0	357·08	0·220	3·780	1 : 17·2

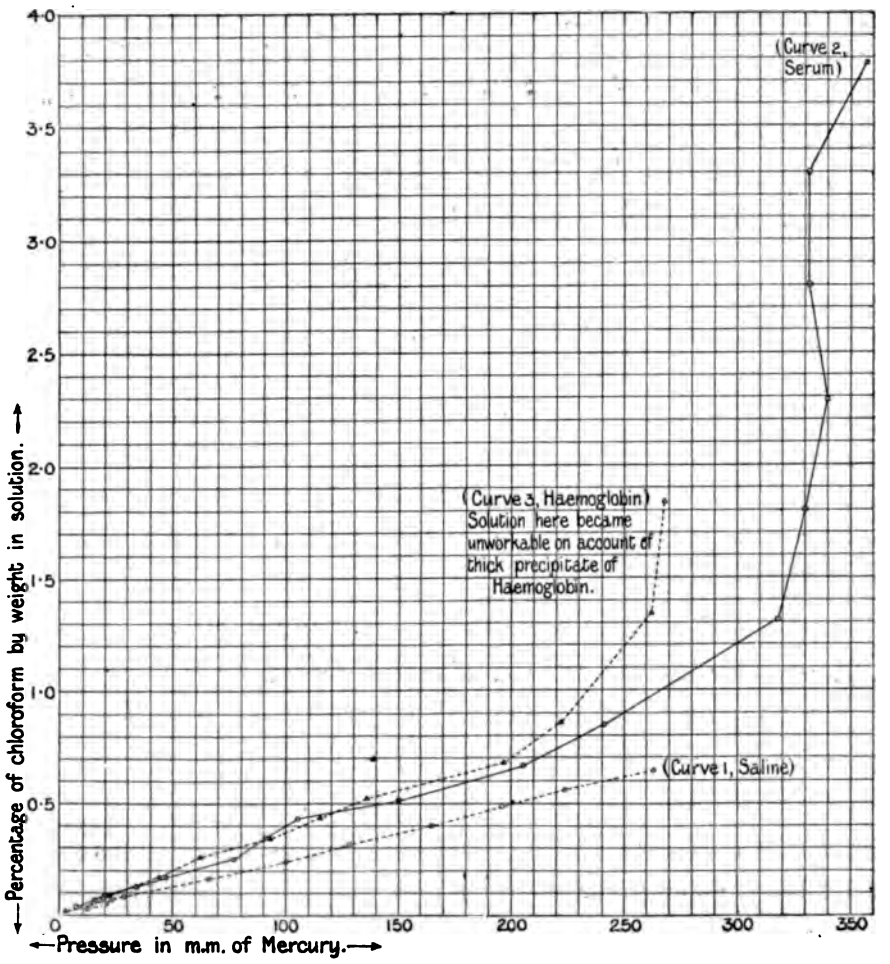
Experiment 7.—Solution of haemoglobin, of equal strength in haemoglobin to blood from which obtained, and containing the amounts of chloroform shown in the table. The results are shown graphically by Curve 3, Fig. 6.

EXPERIMENT VII.—HAEMOGLOBIN SOLUTION

TEMPERATURE 40° C.

Percentage by weight of chloroform originally introduced	Pressure of chloroform in vapour space in mm. of mercury	Percentage by weight of chloroform pumped off into vapour space	Percentage by weight of chloroform remaining in solution	Coefficient of distribution between vapour space and solvent
0·02	2·85	0·0018	0·0182	1 : 10·1
0·04	7·31	0·0045	0·0355	1 : 7·9
0·06	14·52	0·0089	0·0511	1 : 6·6
0·08	17·79	0·0110	0·0690	1 : 6·3
0·1	22·34	0·0138	0·0862	1 : 6·3
0·2	43·85	0·0270	0·1730	1 : 6·4
0·3	62·01	0·0382	0·2618	1 : 6·8
0·4	93·15	0·0573	0·3427	1 : 5·9
0·5	114·91	0·0707	0·4393	1 : 6·2
0·6	136·47	0·0840	0·5160	1 : 6·2
0·8	197·10	0·1213	0·6787	1 : 5·6
1·0	222·64	0·1371	0·8629	1 : 6·3
1·5	262·26	0·1614	1·3386	1 : 8·3
2·0	268·00	0·1650	1·8350	1 : 11·1

FIG. 6


4·0
3·5
3·0
2·5
2·0
1·5
1·0
0·5
0
50
100
150
200
250
300
350
(Curve 2, Serum)
(Curve 3, Haemoglobin) Solution here became unworkable on account of thick precipitate of Haemoglobin.
(Curve 1, Saline)
Percentage of chloroform by weight in solution.
Pressure in m.m. of Mercury.

IV.—Solubilities of Gases in Serum and Haemoglobin in Presence of Chloroform

It was thought that such compounds as are shown by the above experiments to be formed between chloroform and serum or haemoglobin solution might interfere with the carriage of oxygen and carbon-dioxide by the blood. Accordingly, experiments were carried out upon serum and haemoglobin solutions to test this point.

A volume of about 500 c.c. of serum or of haemoglobin solution, obtained as for the experiments in Section 3, was completely deprived of gases by exhaustion with a Töpler pump at 40° C., afterwards saturation with air, or air and carbon-dioxide, was carried out upon two equal volumes contained in similar bottles, to one of which a sufficient quantity of chloroform was added to make a one per cent. solution, while the other served as a control. The gases dissolved in each case were then collected by means of the Töpler pump as before, and analysed.

The results throughout were negative, and thus proved *a fortiori* that at the anaesthetizing values chloroform does not depress the solubility of the respiratory gases in the blood.

As an example, an experiment with serum shaken up with a mixture of air and carbon-dioxide may be quoted :—

A volume of 500 c.c. of serum was exhausted as above described.

(*a*) A volume of 150 c.c. of this serum was poured into a 500 c.c. stoppered bottle, and shaken up with a mixture of air and carbon-dioxide.

Exhaustion and analysis of the gases in 70 c.c. gave the following results at 14° C. and 759 mm. :—

CO_2 41·2 c.c., O = 1·4 cc. N = 5·4 c.c.

(*b*) A second volume of 150 c.c. of the exhausted serum treated exactly similar, but with 1·5 grammes of chloroform added, gave the following results from 70 c.c., at the same temperature and pressure :—

CO_2 41·4 c.c., O = 1·8 c.c. N = 6·4 c.c.

There was obviously a slight leakage of air, but the figures are sufficient to show that there is no appreciable change in the solubilities due to the presence of the chloroform.

V.—On the Solubilities of other Anaesthetics in Water and Serum Respectively

A series of experiments was carried out upon the effects of addition of a number of other anaesthetics to serum, and determinations made of the relative solubilities of these anaesthetics in water and serum.

The anaesthetics so tested were ethyl ether, ethyl acetate, amyl alcohol, amyl acetate, benzol, and xylol.

It was found that the effects with all these substances, without exception, were comparable to those recorded above in detail for chloroform (Sections I and II).

In all cases the maximum solubility in serum is much higher than in water, and at a certain strength of added anaesthetic short of saturation alteration in character of the proteid commences, and finally leads to precipitation. The precipitation commences earlier, and is more complete in some cases than in others. In the case of ethyl ether the precipitation is slight, but the serum becomes gelatinous on standing.

The effects in each case may be briefly summarized as follows :—

Ethyl ether

Solubility in water at 15° C. = 8·0 per cent.
Solubility in serum at 15° C. = 11·0 per cent.

At ten per cent. and over there was a slight opalescence, and the fluid began to grow gelatinous. Eleven per cent. completely dissolved, twelve per cent. not dissolved and at higher percentages, two phases separated, the layer on top forming a clear, thin jelly.

Ethyl acetate

Solubility in water at 15° C. = 7·9 per cent.
Solubility in serum at 15° C. = 10 per cent.

At eight per cent., complete solution of the ethyl acetate and commencing precipitation of the proteid occurred. Solutions of nine and of ten per cent. strengths also dissolved completely, separation into two phases occurred at twelve per cent., the upper layer being viscid.

Amyl alcohol

Solubility in water at 15° C. = 2·4 per cent.
Solubility in serum at 15° C. = 8 per cent. or over.

At three per cent. complete solution of anaesthetic with commencing precipitation of proteid, this precipitation gradually increased through concentrations of 3·5 and four per cent. up to eight per cent., when a thick cream was formed. A separation into two phases could not be observed at any stage.

Amyl acetate

Solubility in water at 15° C. = 0·25 per cent.
Solubility in serum at 15° C. = 1·5 per cent.

At one per cent. complete solution of anaesthetic with precipitation of proteid, at 1·5 all dissolved except a few minute globules.

Benzol

Solubility in water at 15° C. = 0·15 per cent.

Solubility in serum at 15° C. = 0·6 per cent.

At 0·5 per cent. commencing precipitation and opalesence, 0·6 per cent. completely dissolved, 0·7 per cent. not all dissolved.

Xylol

Solubility in water at 15° C. = 0·016 per cent.

Solubility in serum at 15° C. = 0·2 per cent.

At 0·1 per cent. complete solution, cloudy from proteid precipitation, 0·2 per cent. dissolved, 0·3 per cent. not dissolved, 0·4 per cent. not dissolved. It is clear from the above results that a similar action and association between the anaesthetic and the proteid of the serum occurred in each case to that found in the case of chloroform. The amount of ethereal extractive in the different samples of serum used for the determinations varied between 0·24-0·36 per cent., thus demonstrating (see p. 191) that the increased solubilities found could not be due to solution of the anaesthetic in lipöid or ethereal extractives present in the serum.

Summary of Experimental Results, Discussion, and Conclusions

The experiments recorded above show :—

1. That chloroform and other substances possessing the properties of anaesthetics combine in either a physical or chemical manner with proteids in solution, and in so doing alter the properties of the proteid so that (i) the solution becomes opalescent or turbid, or it may be viscid, according to the anaesthetic employed, the change stopping short of actual precipitation of the proteid ; or (ii) at higher concentrations there may be actual precipitation of the proteid which increases with rise of temperature, and may be either complete or incomplete.

Thus, chloroform, in the case of serum, causes a marked opalescence at concentrations far short of saturation, and at higher concentration a slow precipitation at room temperature (15° C.), and at body temperature, a rapid though incomplete precipitation. In the case of haemoglobin, one and a half to two per cent. of chloroform causes a change of colour and commencing precipitation at room temperature, which becomes almost complete in the thermostat at 40° C., while five per cent. and over causes complete precipitation even at 0° C.

Other anaesthetics have been tested and found to produce similar opalescence or turbidity, and a tendency to precipitation in serum.

2. That the maximum solubility of chloroform and a number of other anaesthetics in serum is much higher than in water or in saline of comparable strength to that of the fluids of the body. Thus, serum at a temperature of 13° C. was found to dissolve almost four per cent. of chloroform, while water dissolved less than one per cent. (0·95), and saline solution dissolved 0·83 per cent.

In the case of blood or haemoglobin solution, it is difficult on account of precipitation to estimate with any approach to accuracy the maximum amount of chloroform taken up, but six per cent. by weight may be added, and if rapidly shaken up is found to disappear so completely that no globules of chloroform can be found among the haemoglobin precipitate on careful microscopic examination. It has also been shown that a number of other anaesthetics have a much higher solubility in serum than in water.

3. That, for the same pressure of the anaesthetic at all concentrations, the amount of chloroform dissolved in serum or haemoglobin solution is considerably higher than in saline or water. Or, put in another form, at the same concentration of the anaesthetic in solution the pressure of the anaesthetic in a vacuum or air space is much less in the case of serum or haemoglobin solution than in the case of saline or water.

This has been shown for chloroform in concentrations varying from below the anaesthetization value to near the saturation point, and it has further been shown that while the curve of pressure and concentration in the case of water and saline is a straight line; in the case of serum and haemoglobin solution it is a curve showing association at the higher pressures.

The coefficient of distribution for chloroform at the anaesthetizing pressure (8-10 m.m.) and at 40° C. is, approximately, 4·6 for saline and 7·3 for serum, and at 15° C. these coefficients become 8·8 and 17·3 respectively.

4. The powers of haemoglobin and of serum for dissolving gases are not appreciably affected by the presence of the anaesthetic.

We believe that the experiments recorded above justify the conclusion that chloroform forms an unstable chemical compound or physical aggregation with the proteids experimented with, and that it is carried in the blood in such a state of combination. Since proteids build up the protoplasm of living cells, anaesthesia must be due, in our opinion, to the formation of such compounds which limit the chemical activities of the protoplasm. At low pressures of the anaesthetic, the compounds are unstable, and remain formed only so long as the pressure of the anaesthetic in the solution is maintained. Such compounds are formed not only by haemoglobin but by serum proteid, and hence the position taken by the anaesthetic in haemoglobin is not that of the respiratory oxygen. This is further shown by the fact that the oxygen-carrying power of haemoglobin is not interfered with in presence of chloroform.

Preliminary experiments with brain tissue and with cardiac muscle, both freed from blood, have demonstrated to us that these tissues possess a similar affinity for chloroform, as shown both by the higher solubility of chloroform in them, and at

lower concentrations by the low pressures of the anaesthetic as compared with solutions of equal strength in water or saline.[1]

Since both haemoglobin and, in a lesser degree, even clear serum contain a small percentage of lecithin and other bodies soluble in ether, it has been suggested to us that the results we have obtained may be due to the solubility of the anaesthetic in these bodies and not to an action upon the proteids, and that the experiments should be repeated with proteids which are entirely free from lipöids.

In reply to this criticism, we would point out that in the first place it is a matter of great experimental difficulty, if not impossibility, to obtain proteids in solution unaltered and at the same time free from all trace of fat or lecithin ; and secondly, that a quantitative consideration of the conditions present in our experiments, as we shall proceed to demonstrate, makes it obvious that the small amount of lipöids present could not possibly account for the solubilities and absorption pressures which we have obtained.

Taking first the question of the preparation of lipöid free proteids, any precipitation with a neutral saline reagent will throw down mechanically the lipöid as well as the proteid, and in any process of extraction with a solvent, such as ether, the proteid becomes gradually altered in its properties, as above described. In the preparation of crystallized haemoglobin, to ensure that no lecithin is also mechanically present, repeated re-crystallization becomes necessary, and, as a result, the properties, including the solubility, of the haemoglobin in water become rapidly modified.

We have hence thought it better to obtain all our quantitative results with unattacked, unaltered proteid solutions, and the portion of our work dealing with the changes in physical properties of the solution, such as opalescence, turbidity, and, at higher concentratious, precipitation,[2] have left us in no doubt that the action is upon the dissolved proteid and not upon lipöids.

A quantitative consideration of the conditions in the solutions experimented upon also makes it very clear that it is the proteids and not the lipöids which are responsible for the low pressures and high solubilities.

The coefficient of distribution of chloroform between olive oil and water, according to OVERTON, is 30-33, and as those who support the lipöid theory use olive oil in their experiments as the representative of the lipöids, it would not be unfair to take as the basis of our calculations the above figure as the coefficient of distribution of the anaesthetic between the lipöids and water. Let us take, however, the higher figure of 40 as representing the coefficient of distribution for chloroform between the lipöids of the plasma and water, and calculate the percentage of lipöid which the plasma must contain in order that it may dissolve the amount of chloroform which we have observed on the assumption that none is dissolved or attacked by the proteids.

1. These experiments are in continuation, and the detailed results will appear in a subsequent paper.
2. The large amount of chloroform in the precipitated proteid is clear evidence that the precipitation is due directly to a combination between the chloroform and the proteid. See Edie, p. 195.

According to the law of distribution, the amounts dissolved by the lipöids and by the water respectively will be proportional to the total amount of each present, and to the coefficient of distribution (40), and if there be x parts per cent. of lipöid and $100 - x$ of water, we have the following equation :—

$$\frac{x \times 40}{(100 - x) \times 1} = \frac{\text{Amount dissolved by lipöids}}{\text{Amount dissolved by water}}$$

$$\text{or } \frac{x \times 40 + (100 - x)}{100 - x} = \frac{\text{Total amount dissolved}}{\text{Amount dissolved by water}}$$

or in 100 c.c. of the serum—

$$\frac{39x + 100}{100 - x} = \frac{\text{Total amount dissolved in 100 c.c. of serum}}{\text{Amount dissolved by the water of 100 c.c. of serum}}$$

Now the solubility in serum is 4 per cent., and in water is 0·95 per cent., and substituting these values we obtain—

$$\frac{39x + 100}{100 - x} = \frac{4}{\frac{100 - x}{100} \times 0{\cdot}95}$$

$$\therefore 39x + 100 = \frac{400}{0{\cdot}95}$$

the value of x obtained from this equation is $x = 8{\cdot}23$. That is to say, in order to explain the solubilities which we have observed upon the lipöid theory, it would be necessary to suppose that the serum we used contained approximately 8·23 per cent. of lipöids.

We have found by experiment that the coefficient of distribution increases as the concentration rises, *for the higher percentages of chloroform*, and lies far above forty. On account of this change in coefficient of distribution, the amount of lipöid necessary to account for the solubilities observed is lower than that derived in the above calculation ; but the amount of ethereal extractive in blood serum as a result of several determinations we have found to be only 0·24 to 0·36 per cent., and this amount is obviously entirely inadequate to account for the solubilities observed.

The same reasoning applies to all the other anaesthetics which we have tested, the increased solubilities cannot be referred to the action of the small amounts of lipöid in the serum, and can only arise from a union of some type between the anaesthetic and the proteid.

ON THE ACTION OF CHLOROFORM ON THE PROTEIDS OF SERUM AND UPON SOLUTIONS OF HAEMOGLOBIN

ON THE ACTION OF CHLOROFORM ON THE PROTEIDS OF SERUM AND UPON SOLUTIONS OF HAEMOGLOBIN

By E. S. EDIE, M.A., B.Sc.

FROM THE BIO-CHEMICAL LABORATORY OF THE UNIVERSITY OF LIVERPOOL

In a research recently published in the *Proceedings of the Royal Society*,[1] Moore and Roaf have drawn attention to the formation of compounds between chloroform and the proteids of the blood, and pointed out that even before precipitation occurs, there is evidence of the existence of such compounds, which at that stage are unstable in character, and depend upon the pressure of chloroform in the blood.

In the present communication the matter has been followed up more completely from the chemical point of view, and a more extended examination made of the precipitate as to its stability and amount of chloroform. The condition of the proteid prior to the stage of precipitation by chloroform alone has also been studied by precipitating the proteid with neutral salt, in this case by saturation with ammonium sulphate. The precipitation of proteid from serum or solutions of haemoglobin was noticed first by Salkowski,[2] who used chloroform as a preservative for these fluids, and it was also noticed by Horbaczewski[3] and Formanek, the latter of whom investigated the matter at some length in 1900.[4] He found no chlorine in the precipitate after drying it at 130° C., but the experiments described in this communication would seem to show that any chloroform in an unstable form of combination must be driven off at that temperature, as a more or less constant proportion of chlorine has always been found in the precipitate.

In these experiments the solutions of any desired strength of chloroform were made either by weighing the chloroform into a tared flask by dropping it from a fine capillary pipette, or by adding an accurately measured volume of pure chloroform to a known volume of the proteid solution.

If a solution of haemoglobin is taken and one per cent. of chloroform added, and the mixture shaken thoroughly, all the chloroform dissolves, and no precipitate

1. Vol. 73, p. 382.
2. *Deutsche Med. Wochensch.*, 1888, No. 16, *Zeitsch. f. Physiol. Chem.*, vol. 31, 1900, p. 329.
3. Quoted by Formanek, *loc. cit.*
4. *Zeitsch. f. Physiol. Chem.*, vol. 29, 1900, p. 416.

forms on standing either at room or body temperature. If the percentage of chloroform exceed one-and-a-half, a precipitate is formed more or less readily according to the concentration of the chloroform, and also to the temperature, the precipitate being formed more readily at about 37° C. than at the room temperature.

The highest concentration of chloroform in these experiments with haemoglobin solutions was 7·5 per cent. In this case a copious brick-red precipitate was produced, mixed with a little undissolved chloroform. Before this precipitation occurs, the solution also changes colour and becomes opaque. The precipitate was removed by centrifugalizing, and was washed several times with distilled water. On first centrifugalizing, the precipitate settled down very rapidly, and left the fluid above clear and practically colourless. This fluid was examined for proteid, but only a trace was found, showing that the above concentration of chloroform is sufficient to remove all the proteid from solution.

The nature of this precipitate was next investigated. It was thought that the proteid was, perhaps, simply thrown out of solution by the chloroform in an unchanged form, and experiments were carried out with a view to finding whether the proteid could be got into solution again.

After the precipitate had been thoroughly washed, to get rid, as far as possible, of any mechanically adherent chloroform, it was warmed to about 40° C. with distilled water under the reduced pressure of a water-pump. Even at the ordinary temperature a considerable volume of gas was seen to bubble off, and on warming it came off very vigorously for a time. When there was no further appearance of any gas coming off, the mixture of liquid and precipitate was filtered. The filtrate showed the spectrum of oxy-haemoglobin. This led to the idea that a considerable quantity, or perhaps the whole, of the precipitate might be made to redissolve, and the remainder of it was thoroughly shaken up with water and the liquid examined, but no further action had taken place. This experiment was repeated several times, the various operations being carried through as rapidly as possible, but no further success was obtained in the efforts to get the proteid into solution again after pumping off the chloroform. The precipitate obtained on adding excess of chloroform to haemoglobin solution, after being washed several times, was found to dissolve readily in sodium hydrate, and also in a dilute solution of sodium carbonate, in the latter case giving the spectrum of alkaline haematin.

In these earlier experiments it was assumed that the gas which came off on warming the precipitate with water was chloroform, or at least partly so, but no other method was adopted to prove the presence of or estimate the quantity of chloroform in the precipitate, and the experiments were for a time abandoned. After a short time, however, a method was tried, after being tested on aqueous solutions of chloroform of known strength. The method consists in warming the substance under examination on a steam bath with a saturated solution of potassium permanganate in fifteen per

cent. caustic soda, both of these reagents being free from chlorine. The chloroform thus becomes converted into hydrochloric acid, forming sodium chloride. About fifteen to twenty grams of the moist precipitate are taken and warmed with 150 to 200 c.c. of the permanganate solution in a large beaker, the precipitate being mixed up as thoroughly as possible with the liquid by frequent stirring. After warming the precipitate with this solution for six or seven hours the process is complete, and the insoluble matter is filtered off and washed, and the hydrochloric acid estimated in the filtrate by silver nitrate after neutralizing the excess of alkali with nitric acid.

In the case of haemoglobin solutions, where the amount of chloroform added was insufficient to cause a precipitate, the proteid was thrown out of solution by saturation with ammonium sulphate. The precipitate was then filtered off and washed with a saturated solution of that salt, after which it was treated with the alkaline permanganate solution, and the hydrochloric acid estimated in the usual way.

In the case of serum, obtained by centrifulgalizing after allowing the blood to clot, it was found that it would take up about five per cent. of chloroform. The serum becomes very opalescent, even when considerably smaller percentages of chloroform are added.

On centrifugalizing after saturation of the serum with chloroform, no precipitate settled out even after three-quarters of an hour, the fluid remaining practically as opalescent as at first. If more chloroform was added than the serum could take up, on centrifugalizing, the excess of chloroform was precipitated, mixed with only a very small quantity of proteid. It was therefore found necessary, in the experiments with serum, after allowing it to take up as much chloroform as possible, to precipitate the proteid by saturation with ammonium sulphate. Before doing so, however, the undissolved chloroform was removed by centrifugalizing. The precipitated proteid, after being filtered off, was washed with a saturated solution of ammonium sulphate, and the chlorine determined in the same way as in the precipitate from haemoglobin.

The following shows some of the chief results in the case of haemoglobin solutions :—

Percentage of haemoglobin in solution	Percentage of chloroform added	Percentage of chloroform in precipitate to haemoglobin in solution
13·6	0 5	1·55
11·5	1	1·6
12·3	2	1·3
11·9	3	1·7
15·4	7·5	2·2

The high percentage of chloroform in the precipitate in the last result is doubtless due to the difficulty in washing away all the excess of chloroform. In the other experiments all the chloroform would be taken up, Moore and Roaf finding that[1] solutions of haemoglobin will take up over 6 per cent. by weight of chloroform.

These results, considering the nature of the precipitate, seem to show that the amount of chloroform taken up in precipitating the proteid from haemoglobin solutions is constant, even though widely different percentages of chloroform are added, and, therefore, apart from other facts, it would seem that the precipitate obtained on adding chloroform to haemoglobin solutions is a definite compound or at least a physical aggregation.

The experiments with serum are more troublesome to carry out, owing to the necessity of saturation with ammonium sulphate, and the more thorough washing in order to remove the sodium chloride. The results, however, point to a more or less constant proportion of chloroform in the precipitated proteid.

Percentage of chloroform added to serum	Percentage of chloroform in precipitated proteid to proteid in the serum
0·5	2·4
1	3·6
2	10·9
3	12·3
5	12
6	13·5
8	16

These figures seem to show that the proteid is not fully saturated with chloroform until the amount of the latter added is between two or three per cent., the amount of chloroform in the precipitate with lower percentages being approximately proportional to the amount of chloroform added to the serum.

As serum only takes up about five per cent. of chloroform, the results with six and eight per cent. are probably too high, owing to the difficulty in washing away all the adherent chloroform.

An interesting fact was noticed bearing on the stability of the compound between chloroform and haemoglobin. If the latter solution is first saturated with carbonic oxide, and then 7·5 per cent. of chloroform is added, the solution retains the colour of carbonic oxide haemoglobin, but only a very small precipitate, if any, is formed, and with, say four per cent. of chloroform, no precipitate appears even on long standing. This would seem to prove that chloroform forms a compound which is less stable than carbonic oxide haemoglobin, or at least is not formed in the presence of excess of carbonic oxide.

1. *Loc. cit.*

A SIMPLE METHOD FOR THE PREPARATION AND DETERMINATION OF LECITHIN

A SIMPLE METHOD FOR THE PREPARATION AND DETERMINATION OF LECITHIN

BY
HERBERT E. ROAF, M.B., TORONTO
JOHNSTON COLONIAL FELLOW, UNIVERSITY OF LIVERPOOL
AND
E. S. EDIE, M.A., B.Sc., EDINBURGH

THE majority of the methods which have hitherto been described for the isolation of lecithins from the tissues are based on the method originally designed by DIAKONOW,[1] and modified by HOPPE-SEYLER.[2] This consisted in removing the fats by repeated extraction of the fresh material with ether until the last ethereal extract possessed only a slightly yellow colour. The residue of material after extraction with ether was next extracted with absolute alcohol at 50-60° C., and the filtered extract was evaporated down to the consistency of a syrup, which was taken up in ether, decanted, filtered, and the filtered ethereal solution was evaporated down. The residue from the ether was next dissolved in as small a quantity of absolute alcohol as possible, and the filtered solution exposed for some hours in a freezing mixture to a temperature of 5-10°, when the lecithin was precipitated out of solution.

In this method there is an enormous loss of the available lecithin, not only in the first washings of the tissue with ether, for lecithin has quite a high solubility in ether, but also in the failure of a large part of the lecithin being thrown out from the cold alcohol in the freezing mixture.

While the method was therefore useful in obtaining a small yield of comparatively pure lecithin for purposes of investigation, it is extremely wasteful, and is useless as a method of determination of the amount of lecithin in any given tissue. So much so that most observers have been content to estimate the percentage of lecithin from determinations of the amount of one of its constituents, usually either the phosphorus as magnesium pyrophosphate or the neurin as the platinum salt.

As a means of combating the loss in the ether washings, GILSON[3] recommended that the united ethereal extracts should be separated from the ether by distillation,

1. Hoppe-Seyler's *Med. chem. Untersuch*, 1867.
2. *Handbuch d. physiol-u-path. chem. Analyse*, Berlin, 1893, S. 84.
3. *Zeitsch f. physiol. chemie* (1888), Bd. xii, S. 588.

dissolved in petroleum ether, shaken up in a separating funnel with seventy-five per cent. alcohol twice, after which the alcoholic extracts were united, separated from petroleum ether by distillation of the latter, and allowed to stand in the cold when cholestearin separated out. The alcoholic solution containing the lecithin was separated from the precipitated cholestearin and brought down to a syrup at a temperature of 50-60° C. This syrup was taken up with ether, decanted, filtered, and the ether evaporated off, leaving behind almost pure lecithin. A great advance was made in the methods of separation of lecithin by ZUELZER,[1] who introduced precipitation by acetone in ethereal solution as a means of separation. The acetone precipitates the lecithin but leaves the fats and cholestearin in solution. ZUELZER, however, still retained the practice of repeated extraction of the fresh material with ether, and precipitated the lecithin from the united ethereal extracts. It is obvious from the original method of obtaining lecithin, in which these ethereal extracts were neglected, and still a yield of lecithin obtained from the residue on subsequent treatment with alcohol, that the initial extraction of the fresh tissue with repeated quantities of fresh ether is a slow, laborious, and incomplete process[2] for the removal of lecithin from tissues containing it, and quite unsuited for determination of the amount of lecithin in a given tissue.

BERGELL,[3] instead of extracting with ether, used alcohol, and instead of precipitating with acetone, used an alcoholic solution of cadmium chloride as a precipitant in the alcoholic solution of the lecithin, and afterwards broke up the cadmium precipitate by boiling with alcohol and ammonium carbonate.

The yield obtained by BERGELL by this method was, however, very low, amounting for egg yolk to about four per cent., which is less than half that obtained by the method to be described in this paper. Also experimentation with alcoholic solutions of pure lecithin and precipitation with cadmium chloride has proved to us that cadmium chloride is not a complete precipitant of lecithin, a result which confirms the observations of SCHULZE and WINTERSTEIN,[4] and shows that this salt cannot be used for complete precipitation or estimation of lecithin. On the other hand, we have found that precipitation by acetone in ethereal solution is practically complete, and in absence of excess of fats and cholestearin, and following on a more complete method of extraction from the tissue than is extraction with ether, can be made to form the basis for the extraction, separation, and estimation of the entire lecithin.

A consideration of the solubilities of lecithin on the one hand, and of the bodies present along with it in the tissues from which the problem is to separate it, shows that extraction with ether is a bad start to make with the fresh tissue. In the first place, the fluid in the tissues is water, or rather saline, which does not mix with

1. *Zeitsch f. physiol chemie* (1899), Bd. xxvii, S. 255. Altmann first recommended precipitation of lecithin by acetone from concentrated chloroform solutions. Quoted from Hoppe-Seyler's *Handbuch der physiol. u. pathol. chem. analyse* (1893), S. 84.
2. See also E. Schulze u. E. Winterstein, *Zeitsch. f. physiol. chemie* (1903), Bd. xl, S. 109.
3. *Ber d. deutsch. chem. Ges.* (1900), 33, SS. 2584-2586.
4. *Loc. cit.*

ether, and hence for any organized tissue the latter solvent has a poor penetrating power, and long continued and repeated extraction is necessary in order to make certain of complete extraction of any lecithin which may be free in the tissue.

Secondly, it has been shown that lecithin, in part at least, exists in combination with albumin forming the so-called lecith-albumins, and it has been shown that the lecithin in these is not dissolved out by ether even on repeated extraction.

Thirdly, no separation occurs in the extraction with ether, because all the bodies concerned, viz., fats, cholestearin, and lecithin, are all easily soluble in ether, and hence the ethereal extract is loaded up with fats and cholestearin which are present in such large comparative quantity that it becomes afterwards exceedingly difficult to purify.

Alcohol possesses the advantage over ether as a first extracting agent, in that it takes up the water of the tissue and penetrates to all parts, it also coagulates the proteid, and hence breaks up the lecith-albumins and allows the whole of the lecithin to be extracted.

A consideration of the solubilities of the fats, cholestearin, and lecithin in alcohol at different temperatures indicates that a considerable amount of separation of the lecithin from the fats and cholestearin may be effected, in the first instance, by using the alcohol at ordinary atmospheric temperatures (10-20° C.), instead of as has usually been done, employing the alcohol at a temperature of 40-50° C.

All the bodies concerned are readily soluble in alcohol at the higher temperatures, but at room temperatures the oils and cholestearin are only sparingly soluble, while lecithin still possesses a high solubility, and does not begin to pass out of solution in large amounts until the temperature falls below 0° C.

Thus, when a fat or oil is treated with alcohol at room temperature only a small amount dissolves, but on heating the fat passes into solution readily, and on cooling the excess is again thrown out. Cholestearin also does not dissolve appreciably in alcohol at 10-15° C., but on heating dissolves readily, and crystallizes out again in great part on cooling. Lecithin, on the other hand, dissolves quite readily in alcohol at room temperatures.

We have used this in conjunction with acetone precipitation as a method of separating and estimating lecithin, and have found it to yield good results.

The method in outline may be described as follows :—The material to be tested (egg yolk was used in our experiments) is weighed and extracted with three to four times its weight of cold alcohol (methylated spirit of ninety-five per cent. or absolute alcohol), each quantity of alcohol being left for two or three hours in contact with the material. Each time filtration through linen is used for separating the alcoholic extract, and at the end the three clear extracts are united and the alcohol evaporated off preferably in vacuo until a syrup is obtained. The syrup is taken up in a small volume of ether and precipitated with excess of acetone added as long as a precipitate is obtained. The lecithin is completely thrown out of solution, as is shown by a

phosphorus determination in the residue obtained by evaporating off the ether and acetone from the mother liquor. But the lecithin thrown out by the first precipitation as shown by a phosphorus determination is impure, and must be purified by re-solution in a small volume of ether and reprecipitation by acetone, the loss of lecithin in the process of reprecipitation is negligible, and determination of the phosphorus in the reprecipitated lecithin shows that it is pure enough to allow of determination of the lecithin by direct weighing as such.

The method of isolation accordingly gives a higher yield than former methods, on account of the more complete extraction and of very small loss in the subsequent precipitation, and the amount of labour involved is much decreased.

Experiment 1. The yolks of six eggs were separated and weighed; weight 108·82 grammes. The yolks were well mixed with 300 c.c. of absolute alcohol, allowed to stand for some hours, the solution decanted, and the rest of the alcohol pressed off as completely as possible through linen cloth. The treatment was repeated with a second and a third quantity of 300 c.c. of alcohol. The three alcoholic extracts were united and the alcohol distilled off under diminished pressure, until a thick syrupy mass was obtained. This was twice dissolved in as little ether as possible, precipitated with acetone, then separated and dried from ether and acetone, in a steam bath to a constant weight of 10·38 grammes, percentage of lecithin, 9·5 of the total weight of fresh egg yolk.

Experiment 2. Carried out similarly. Weight of egg yolk = 120·18 grammes; yield of lecithin, 11·02; percentage of lecithin, 9·1.

In order to determine whether the lecithin is completely precipitated by the acetone from ethereal solution, the mixture of ether and acetone separated at each precipitation was evaporated down. The residue was extracted with alcohol in the cold, thus leaving behind most of the cholestearin. The process of extraction with alcohol was several times repeated, the extracts mixed and evaporated down. A very small amount of a brown oily fluid was obtained, which was treated by baryta water and filtered. The filtrate was treated with dilute sulphuric acid and the barium sulphate filtered off. The free glycero-phosphoric acid was, after evaporation to dryness, ignited with fusion mixture, and phosphoric acid estimated as magnesium pyrophosphate. The weight of $Mg_2P_2O_7$ obtained was only 0·0184 gramme, showing that practically none of the lecithin had escaped precipitation by the acetone. The barium soaps were also investigated by breaking up with dilute sulphuric acid, formation of lead soaps, separation of lead soaps, and determination of amounts of fatty acids, and only inappreciable traces were found.

The purity of the yield of lecithin obtained was investigated by making determinations of the phosphorus, by a procedure which, when we first used it, we thought to be novel, but afterwards we discovered that it was similar to that described by NEUMANN[1] for determination of phosphorus in nucleo-proteid, and later by

1. *Archiv. f.* [*Anat. u.*] *Physiologie* (1900), S. 159.

MALCOLM[1] in egg yolk. The method is an excellent one for the determination of phosphorus in lecithin, and consists in carrying out a modified Kjeldahl determination, in which, however, phosphorus is determined as magnesium pyro-phosphate, instead of nitrogen as ammonia.

About 1·5 gramme of lecithin is taken and boiled in a Kjedahl flask with pure concentrated sulphuric acid, potassium sulphate being added from time to time to assist in the oxidation, when a colourless fluid is obtained it is allowed to cool neutralized by ammonia and the phosphoric acid estimated gravimetrically as $Mg_2P_2O_7$.

Experiment 3

	Lecithin taken	$Mg_2P_2O_7$ obtained	Percentage of Phosphorus	Estimated molecular wt. of lecithin
Determination 1 ...	2·752	0·3915	3·973	780
,, 2 ...	1·663	0·2362	3·966	782
,, 3 ...	1·541	0·2170	3·933	788

The molecular weight of di-stearyl-lecithin is 807, di-palmityl-lecithin is 751, di-oleal-lecithin is 803, and of di-linoleal-lecithin is 743. According to COUSINS,[2] who separated and determined the fatty acids in lecithin of egg-yolk, this contains linoleic acid, 24·0 per cent.; oleic acid, 33 per cent. ; palmitic acid, 28·5 per cent. ; and stearic acid, 14·2 per cent. This composition would lead to a mean molecular weight of 772, which fairly closely corroborates the above results.

1. *Journ. of Physiol.* (1901), vol. xxvii, p. 355.
2. *Soc. Biol.* 55, p. 913

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THE

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EDITED BY

RUBERT BOYCE AND CHARLES S. SHERRINGTON

WITH

H. E. ANNETT, BENJAMIN MOORE, RONALD ROSS AND E. W. HOPE

ASSISTED BY

W. B. WARRINGTON, E. E. GLYNN

J. W. W. STEPHENS, J. L. TODD, J. WOLFERSTAN THOMAS, ANTON BREINL

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ON

TRYPANOSOMES, TRYPANOSOMIASIS, AND SLEEPING SICKNESS

BEING

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BY

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AND

A DESCRIPTION OF THE TISSUE CHANGES

BY

ANTON BREINL, M.U. DR. (*Prag.*)

J. W. GARRETT INTERNATIONAL FELLOW

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OCTOBER, 1905

In Memoriam

Jacob Hunter Todd

Died, August 10, 1899, Victoria, B.C., Canada

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PREFACE

THE experimental work on Trypanosomiasis which the Liverpool School of Tropical Medicine has undertaken owes its inception to the discovery by DUTTON in 1902 of a trypanosome in the blood of a European under the care of Dr. FORDE. Drs. DUTTON and TODD were appointed by the School to investigate the disease in Senegambia. The expedition returned in the summer of 1903, bringing with them animals infected with *Trypanosoma gambiense* from natives suffering from the disease in Senegambia, and also animals infected with *Trypanosoma dimorphon* (Gambian Horse Disease). As Drs. DUTTON and TODD had to leave in September, 1903, for the Congo Free State the School decided to carry on the research. On my taking charge of the work I was assisted by Dr. S. F. LINTON, who continued with me until June, 1904. Dr. ANTON BREINL (J. W. Garrett International Fellow) was then invited to undertake the pathologico-histological side of the work. He has been given opportunity of studying and comparing material from three cases of sleeping sickness and one case of trypanosome fever in man, together with material from a large number of animals dying from the various trypanosomic diseases.

The following gentlemen have aided the research in blood-counting observations on the animals:—Dr. R. E. MCCONNELL, Mr. P. A. RADCLIFFE. Special analyses of urine were made by Mr. EDIE (Bio-Chemistry Department). In addition to studying *T. gambiense* and *T. dimorphon* other pathogenic trypanosomes were procured, so that a comparison could be made between the above micro-organisms and *T. evansi*, *T. brucei*, *T. equinum*, *T. equiperdum*.

Extensive experiments have been made with various drugs to cure the disease. Observations have been made upon serum and agglutination work.

The experimental trypanosomiasis research has embraced such a large number of problems that my colleague and myself have been unable to complete all the lines of work. The experimental work has naturally depended largely upon the effects of the disease in animals, and frequently the labour of months has had to be repeated by reason of epidemics amongst the inoculated animals.

The report is not so complete as wished for, but the immediate departure of Dr. BREINL and myself on an expedition to Brazil has compelled us to give our results in the manner here presented. We hope to fill in the omissions at an early date.

In conclusion I wish to acknowledge, on behalf of the research workers, the courtesy and interest which so many have evinced in the work. In particular Professors BOYCE, Ross, SHERRINGTON, and MOORE, Dr. J. W. W. STEPHENS, Dr. J. HILL ABRAM, Dr. LLOYD ROBERTS, Professor GRÜNBAUM, and Dr. ERNEST GLYNN, Dr. LAUDER, Medical Health and Port Sanitary Officer, Southampton, his assistant, Dr. MCCULLOCH, and the Matron of the Southampton Isolation Hospital.

I cannot close without expressing my indebtedness to Dr. H. E. ANNETT, who has done everything possible to aid the research department, to him and Professor BOYCE the numerous worries and difficulties associated with the work have been left.

To the gentlemen on the committee and the Hon. Secretary of the School, who have endeavoured to meet all the requirements of the division, our thanks are tendered.

On behalf of the workers

H. W. THOMAS

CONTENTS

ERRATA AND ADDENDA

Page 4, line 12, *read* 'especially right inguinal and left cervical.'

Page 4, line 20, 'electrical reaction,' *read* 'electrical resistance.'

Page 7, Early history of Banja, Memoir XIII, L.S.T.M., p. 41.

Page 13, reference to Dutton and Todd, p. 97 of this volume.

Page 17, line 35, *read* 'small quantities of blood from an infected animal failed to produce infection.'

Page 30, Horse ; this is the animal referred to as 'Horse VI.'

Page 48, line 16, 'evidences,' *read* 'evidence.'

Page 49, line 4, 'Governors,' *read* 'Governors'.'

Page 49, line 8, 'Laver,' *read* 'Laveran.'

Page 49, line 10, 'potassium, and,' *read* 'potassium and.'

Page 50, line 14, 'Fowelri,' *read* 'Fowleri.'

Page 53, line 39, '889,' *read* '839.'

Page 59, line 4, 'are seen after,' *read* 'are seen but after.'

Page 60, line 20, 'subjects,' *read* 'substances.'

Page 62, line 24, '*P. rodigosus*,' *read* '*B. prodigiosus*.'

TRYPANOSOMES, TRYPANOSOMIASIS, AND 'SLEEPING SICKNESS'

BY
H. W. THOMAS
J. H. TODD MEMORIAL FELLOW
AND
ANTON BREINL

I. DESCRIPTION OF CASES OF SLEEPING SICKNESS IN MAN

OPPORTUNITY was afforded for study by the arrival in England of natives from the Congo Free State suffering from 'sleeping sickness' or 'Trypanosome Native Fever.' It is due to the courtesy of the authorities of the Congo Free State that facilities were given to the members of the Congo Expedition of our School to send to England selected cases of the disease. Special importance was placed on observations being made for a prolonged period in England where the change of climate, the regular life, and the general attention given to strengthening their systems might produce some effect on the disease. The cases observed were :—

Of 'Sleeping Sickness'	Of 'Trypanosomiasis' ('Trypanosome Fever')
Boyo	Mpangila
Kitambo	Disasi
Tomi	Banja
	One European Capt. 'S. B.'

It is not our purpose to discuss clinically whether 'Trypanosome Fever' cases are cases of early sleeping sickness or represent a distinct disease—this is left to the members of our Congo Expedition who have had opportunity of comparing several hundreds of cases. All the sleeping sickness cases died, the native 'Disasi' succumbed to pneumonia. Clinical histories of cases of trypanosomiasis in man have been so frequently recorded that a minutely detailed report of each case would serve no purpose.

Kitambo. Male, *aet.* 23. A native of Nyangwé.

History: Three years in Leopoldville. Was ten days in prison before coming to hospital. In prison felt ill and had pains all over the body. Could do no work, because of giddiness, headache, and weakness.

Condition. March 28, 1904: Nutrition fair. Eyes heavy. Intelligence fair. Complains of frontal headache. Tremors of lips and hands. Gait steady. Skin moist. Glands, except epitrochlear, enlarged. Oedema of feet and shins.

Heart: Pulmonary second sound accentuated, rough and reduplicated; apex in nipple line in sixth interspace.

Spleen enlarged.

Liver normal.

Lungs normal.

Nervous System: Knee jerks normal. Cremasteric reflex increased. Pupil reacts to accommodation and light. Tongue very unsteady.

April 15: Sleeps a great deal in daytime. Unsteady gait. Very weak: cries for very little. Eyes sluggish to accommodation and light, pupils small. Tremor of legs when standing. Knee jerks and superficial reflexes increased. Nystagmus marked. During the voyage to England patient continued in about the same state. During the two days in Southampton he dozed most of the day. On arrival in Liverpool, May 24, he was able to walk to the cab. The man was placed in Dr. J. Hill Abram's Ward at the Royal Infirmary.

The following notes are taken from the hospital records by permission of Dr. J. Hill Abram:—

Present condition: Drowsiness and tremors. Lies on his back: not well nourished. Rash all over body. Sleeps a great deal. Slight glandular enlargement in left axilla and neck, and considerable in inguinal regions.

Alimentary System: Teeth good. Tongue tremulous and coated white. Abdomen slightly distended. No enlargement of liver, spleen, or stomach.

Circulatory System: Pulse frequent, soft. Apex beat just perceptible and palpable in fifth space, three inches from middle line. Sounds normal and no hypertrophy.

Respiratory System: Respiration frequent, tenderness and slight oedema over chest.

Urinary System: 2674 grammes, sp. gr. 1025, alkaline, deposit of phosphates, no sugar, no albumen, Ehrlich reaction negative.

Skin: Papular rash (scabies).

Nervous System: Very sleepy, general tremulousness. Pupils small. Knee jerk and plantar reflexes lively.

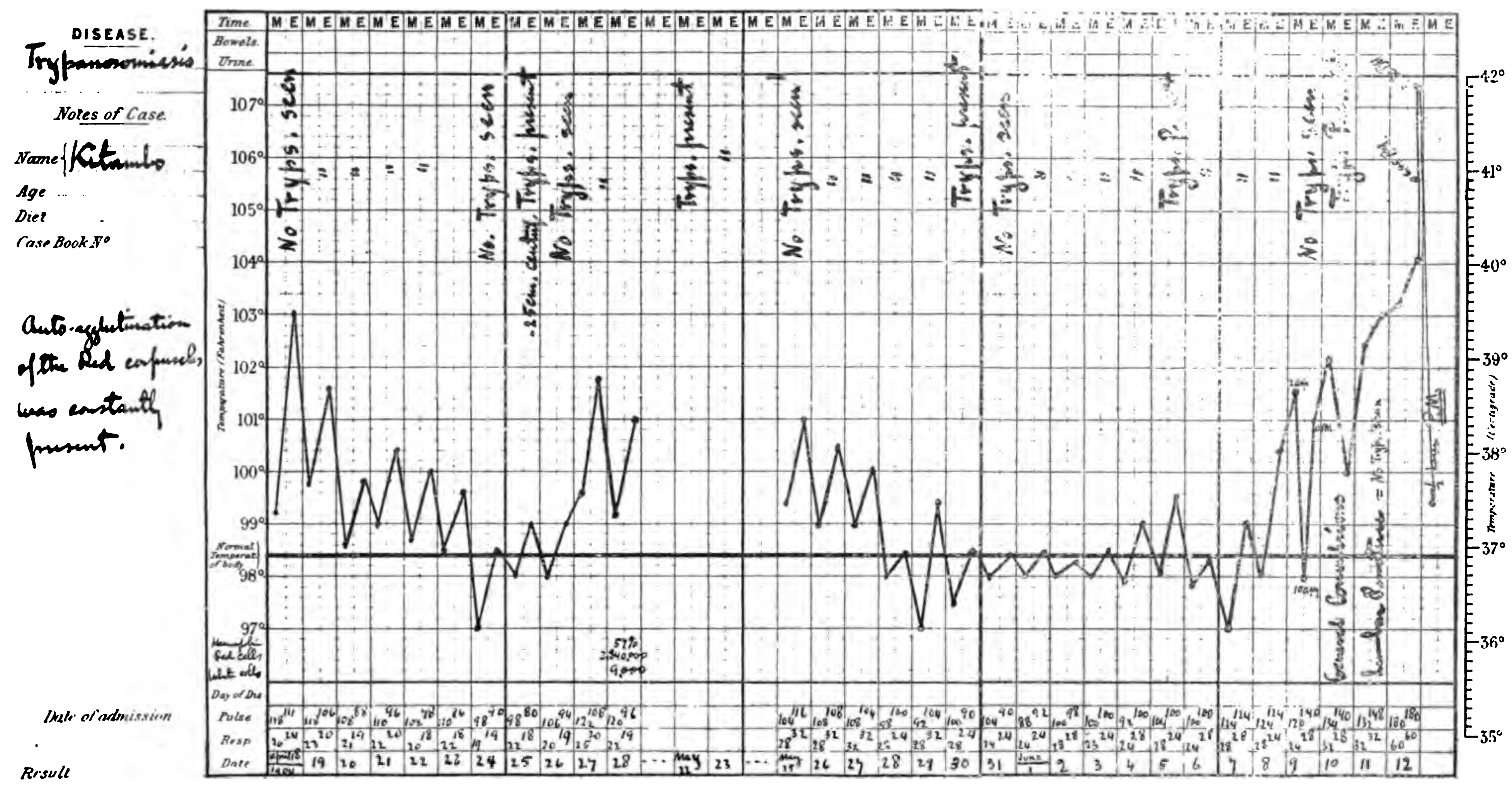
On June 3, 4, and 5, profuse sweating at night.

June 7: Last three or four days seemed weaker. Cannot now feed himself nor walk. Cannot raise himself in bed. Urine and faeces passed involuntarily. Knee jerks present. No increase in drowsiness. Heart and lungs clear. Pulse, 108, softer. Occasional twitchings with considerable asthenic tremor in hands. Pupils small; takes food readily, but has to be fed. Blood, haemoglobin, 65 per cent., red blood cells, 3,720,000, white blood cells, 7,812. Eosinophiles in comparison with the rest, not nearly so numerous as on May 25.

June 9: Conjunctivae injected and muco-purulent discharge.

June 10, 2.50 to 3.15 a.m.: General convulsions, three fits, last fit continued for about ten minutes. Conjugate deviation of eyes and head to right. Both arms and legs worked, but right arm seemed to work more than the left, and movement was flexion and extension at elbow. Pulse 170.

CHART I



to face p. 2

CHART II

DISEASE.

Trypanosomiasis

Notes of Case.

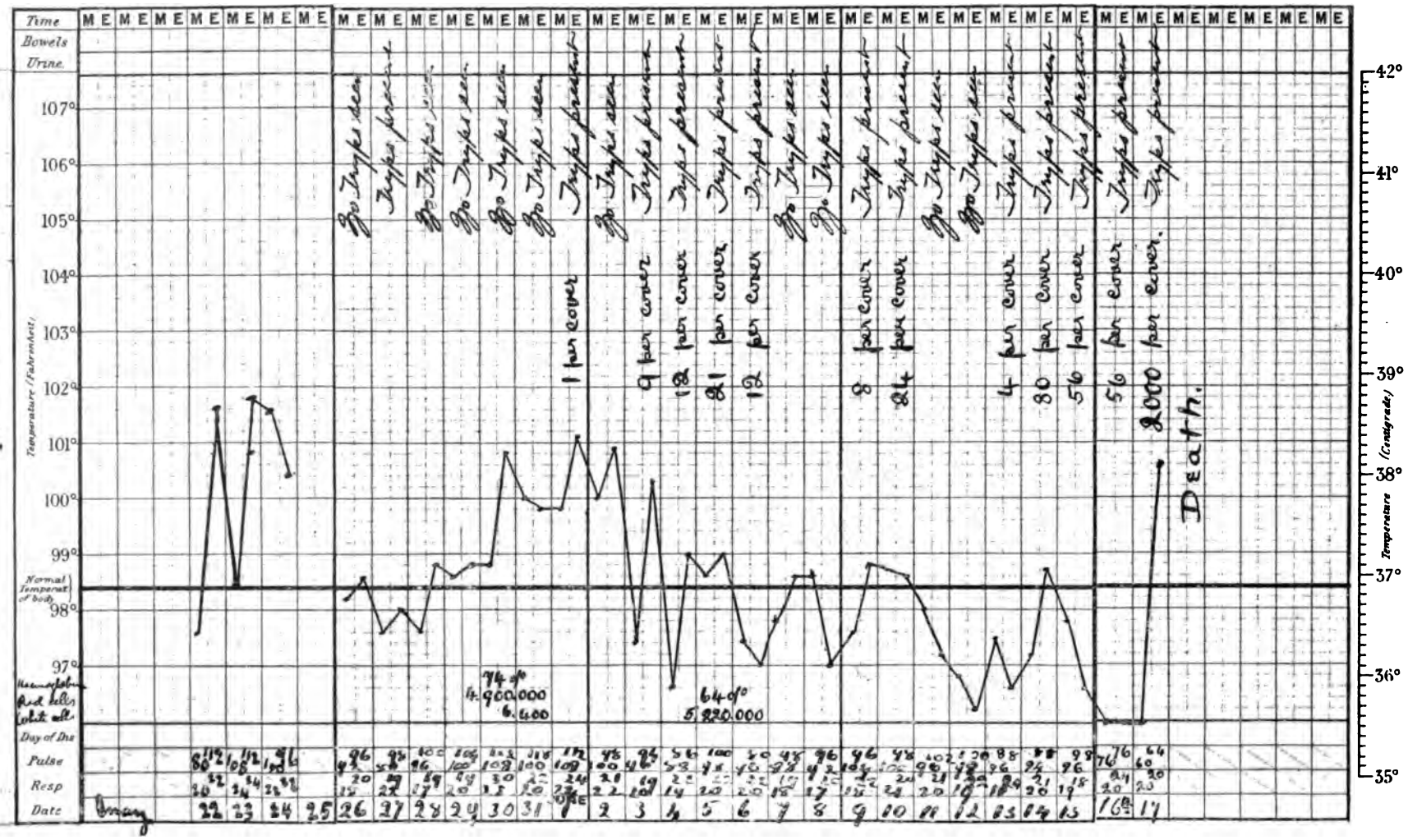
Name { Tomi

Age

Diet

Case Book N°

autoagglutination of Red cells constantly present.



Date of admission Aug 22/05

Result

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7.30 p.m. : Unconscious most of day. At times seemed to hear when spoken to, but most of the time no response. Bowels moved from enema 2.30 p.m. and acted several times since. Croton oil, mid-day. Just had two general convulsions, lasting about three minutes each. Last fit, head and eyes first to left, afterwards to right. Arms and legs worked too. (Whilst writing a third fit came on). Conjunctivae much injected, good deal of muco-purulent discharge.

June 11, *midnight:* Semi-comatose during last twenty-four hours. No fits ; takes milk and whiskey when given. Corneal reflex present. Winced slightly when needle pierced skin for lumbar puncture to-day. No fluid ran out, a little drawn out by syringe, but mixed with blood. Profuse sweating on face, forehead, and neck. Past forty-eight hours, not much sweating on trunk. Pulse, 145, full, low tension. Respiration, 32 ; urine and faeces passed involuntarily.

June 12, 6.45 *p.m.* : Temperature getting higher all day and now 105·6. Pulse, 200, small volume, poor tension. Head and neck sweating still, trunk not. Respiration, 48 ; alae nasi working ; Cheyne-Stokes in type at times. Not been able to swallow fluid food to-day.

7.30 *p.m.* : T. 107·4 (thermometer seen).

7.45 *p.m.* : *In extremis;* face covered with cold perspiration. Corneal and knee jerk reflexes gone. T. 105·8. Heart beats 168 at apex, every third or fourth only reaching wrist. Respiration, 24, jerky (chin could be made to touch sternum).

7.55 : Respiration, 8 per min. Heart, 56. Violent last inspiration. Heart and respiration failed together. No P.M. rise of temperature, but fall (see chart).

For autopsy and morbid anatomy see Part II, Kitambo.

Tomi. *Aet* 18. Male.

He had been employed as cook to the Rev. Mr. Gordon at the Kinshasa Mission of the Baptist Missionary Society, Stanley Pool. As he was only seen a couple of times by the members of the Expedition, no detailed history is procurable, Mr. Gordon first noticed the boy to be careless, disinterested, and quarrelsome in December, 1903. In January, 1904, he complained of pains in one eye, which became inflamed. It improved for a time, but by the end of February both eyes were affected. This, coupled with the increasing irritableness and his being found sleeping at odd times in the cook house caused the missionaries to fear he had 'manimba.'[1] In April he was examined by Dr. Dutton, and parasites found.

On his arrival at Southampton, May 22, he was able to walk, but was extremely nervous and hysterical, laughing and crying without cause. Marked tremor of the upper and lower limbs when in a sitting or recumbent posture was observed. This condition was accentuated if the boy noticed anyone looking at him. On his arrival in Liverpool, May 24, he was very excited, and homesick, but became quiet on being given light work to do.

General Condition : A rather emaciated averaged-sized boy. Muscular development poor. Skin soft and clean—chiggers in both feet. Glandular enlargement general but not so marked as in other cases. Gait unsteady and very irregular, this is due to the chiggers. On standing still with the eyes closed he remains motionless for a few minutes and then starts to sway ; power over the knees suddenly appears to be lost and he will fall ; he can stand motionless if allowed to keep his eyes open, but after a while sways from weakness. At times, if seated, a violent tremor commences in the lower limbs, and heels rap the floor ; the knees, especially if the toes are resting on the floor, shake sideways and a little forwards. The hands and arms may remain quiet or start twitching spasmodically. The head usually moves sideways, the lips tremble, the eyelids twitch and the eyes roll. Plaintive sounds are emitted but no coherent words. These symptoms may cease after a few minutes, or a crying fit terminate the attack. If spoken to he can usually rise and appear quite well again, at other times, the trembling, etc., is not interrupted and the general vacant expression continues. Much is due to hysteria. If taken into a dark room and

1. A native name for 'sleeping sickness.'

his attention engaged, no swaying or falling occurs even if the heels are together. At night he will sit quite motionless without trembling. He is fanciful in his wishes and tastes, and very quarrelsome with his companions.

Respiratory system: Lungs normal; expansion good.

Circulatory system: Heart, normal size and position. Slight accentuation of pulmonary sound. Pulse variable, often markedly irregular, quick, without tone, and soft, at other times of high tension with a slow but regular rhythm.

Digestive system: Teeth good; faeces contain ova of *Ankylostoma duodenale.*

Liver not enlarged nor painful.

Spleen moderately enlarged, not painful on palpation.

Genito-urinary system: Urine normal, sp. gr. 1020. No albumen. No sugar.

ANALYSIS OF URINE MADE BY MR. EDIE, LABORATORY FOR BIO-CHEMISTRY

Date	c.c. Urine	Sp. Gr.	Reaction	Gram Total Nitrogen	Gram Urea	Gram Uric Acid	Gram NH_3	
June 17	325	1014	Neutral	3·14	5·82	·11	·05	Albumen and Sugar absent

Glandular system. All groups enlarged, especially right. Inguinal and left cervical freely moveable, not painful.

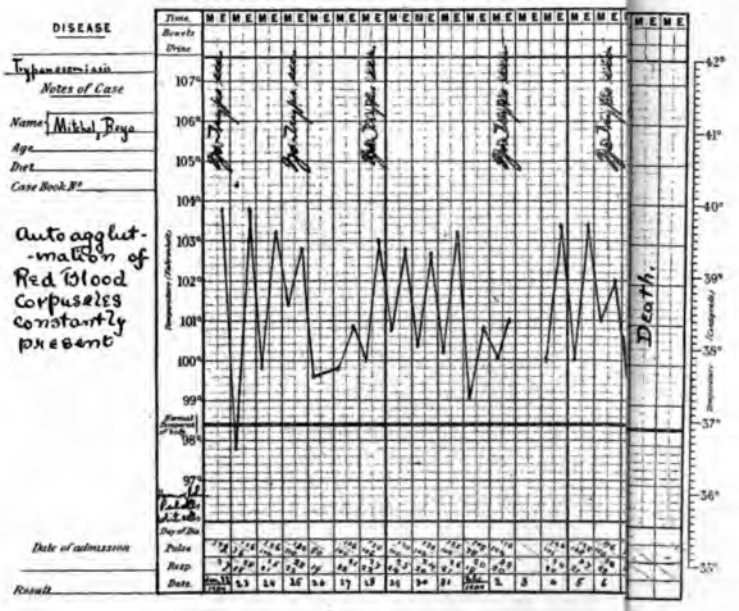
Aural and nasal systems normal.

Pupil not dilated. Reaction to accommodation and light normal. Small opacity on cornea of left eye about 1 mm. in diameter.

Nervous system: Knee jerks increased. Cremasteric, epigastric, and anal reflexes not accentuated. Jaw clonus normal. Ankle clonus very accentuated at times.

Electrical reaction of skin normal. Some of the peripheral nerves reacted very slowly to the galvanic and faradaic currents. Some evidenced the degeneration reaction. The majority reacted normally.

From May 25 to June 11 the boy continued in the same state, seemed to gain in strength and became less excitable. The tremors became less accentuated and he learnt rapidly. At times he would become depressed and say he had 'manimba' and was going to die. *Sleep symptoms were not noticed.* During this period the parasites were always scanty, but appeared to be increasing on the 12th. On that day he finished his work and went to dinner; at 2 p.m. the attendant came and said he could not wake him. He was found lying on his side with his knees drawn up, apparently asleep and perfectly motionless. On being shaken and shouted at he would open his eyes and utter a word and then relapse into the somnolent state. The temperature *per rectum* was 96·2° F. The cremasteric and epigastric reflexes normal, the anal much less. The knee jerk was much lessened. Respiration, 22, full and regular. Pulse, 100, soft, low tension, and irregular. After four hours he woke up and appeared to feel as usual, though somewhat dazed and unsteady in his gait. For the next two days he was kept in bed, but was always getting out and sitting on a chair. Parasites were increasing in numbers. The temperature continued sub-normal. On the 15th, 30 c.c. of cerebro-spinal fluid was drawn off by Dr. CHRISTY without difficulty, the fluid welling out when the needle entered the canal. The clear fluid, not contaminated with blood, contained trypanosomes, as many as five to a field. The blood at this time contained 700 to a cover, irregularly distributed, groups of two, four, and six being


DISEASE
Trypanosomiasis
Notes of Case
Name Mikhul Boya
Age
Diet
Case Book Pt.
Auto agglut-mation of Red Blood Corpuscles constantly present
Time.
Bowels
Urine
Temperature (Fahrenheit)
Pulse
Resp.
Date.
Day of Dis.
Date of admission
Result
Death.
Temperature (Centigrade)

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seen. Part of the cerebro-spinal fluid was inoculated into a monkey, two rats, and a rabbit. The boy was kept in bed, though he said he felt well. The next day he was found in a semicomatose state. Temperature, 96·4° F.; respiration, 19; pulse, 108. Deviation of the eyes was noted. He was taken to the Royal Southern Hospital, and placed under the care of Dr. Lloyd Roberts. Until he died, on June 17, he remained in a somnolent state. At first nourishment in the form of soup could be given, but later total unconsciousness intervened. There was incontinence of urine and faeces. The temperature was sub-normal, but started to rise on the evening of the 16th, registering 100·6° F. at death. The reflexes were all decreased: sensations dulled. The pupils reacted to accommodation and light. The parasites continued to increase in numbers—a few hours before death two to three to a field were seen.

The differential blood counts made on two different days gave the following results :—

	June 11	June 16
Polymorphonuclear neutrophile ...	77·63	76·00
Large mononuclears	4·15	2·8
Lymphocytes	10·77	11·2
Eosinophiles	4·71	5·1
Transitional forms	2·14	4·2
Mast cells	0·60	0·7
	100·00	100·0
Trypanosomes	Absent	1 to 2 to a field

For autopsy, etc., see Part II, Tomi.

Boyo Mitchel. Male. *Aet.* 14. Coquilhatville.

First seen January 21, 1904. Came to Leopoldville two years ago. First felt ill coming down the Kasai river two or three months since, when he had headaches.

February 5: Thin and wasted. Gait slow, unsteady, and shuffling. Expression dull, apathetic, and vacant. Answers questions slowly and in a weak voice. Very drowsy.

Physical condition: Skin dry and scurfy. Glands enlarged. No oedema. Slight puffiness about eyes and puffy appearance of whole of face. Mouth full of sores.

Heart and Lungs normal.

Spleen not enlarged.

Liver apparently enlarged.

Knee jerks normal. Epigastric and cremasteric reflexes normal.

Faeces contain anchylostoma and ascarides.

Up to time of departure for England the boy remained in about the same state. Tremors being marked at times.

May 13: He walked with difficulty to the steamer. Temperature, 104 for two days; patient in state of collapse; could eat nothing. Urine and faeces passed in bed. After two days at sea he revived a little; the temperature fell and he was able to sit on deck. Mouth very foul. Later he was unable to get out of his bunk. Could only take soup and water if fed with a spoon

May 21 : Condition much worse. Emaciation very marked. Motions passed in bed. Bedsores over sacrum, others commencing on ankles, knees, and scapulae. Pulse extremely weak. Extremities cold. Gums ulcerated, teeth loose, mouth very foetid. Treatment of no avail.

May 22 : Taken to Isolation Hospital, Southampton. Condition after two days improved, but death took place May 24. Patient was lumbar-punctured six times, on three occasions parasites were present in the cerebro-spinal fluid. A rat injected with some of the fluid shortly after death, developed the disease in a mild form thirty-five days after inoculation.

For autopsy and morbid histology, see Part II, Boyo.

Mpangila. Male. *Aet.* 17.

Arrived in Liverpool, December 27, 1904. Parasites had been found in the blood in November, 1904, by Dr. Dutton.

Physical condition : A well-developed boy ; muscular development, fair. Thin, but not emaciated. Intelligence, above average. Easily tires ; no tendency to somnolence. Glandular enlargement general. Skin soft. No oedema. No eruptions on skin.

Respiratory system normal.

Circulatory system : Heart sounds, normal. Pulse, 96-104, soft and regular, at times becoming more rapid and irregular, without cause or relation to the temperature, rising to 110 and falling again to 96. This occurred when at rest or during work ; heavy labour did not appear to affect it.

Spleen : Slightly enlarged ; not tender on palpation.

Liver normal.

Genito-urinary system normal. Urine, sp. gr. 1018. No albumen ; no sugar ; chlorides ; no bilirubin. The following analyses were made by Mr. Edie, Laboratory of Bio-Chemistry :—

Date	c.c. Urine	Sp. Gr.	Reaction to Litmus	Grams Total Nitrogen	Grams Urea	Grams Uric Acid	Grams NH_3	Grams NaCl	Grams P_2O_5	Albumen	Sugar
July 9	1100	1015	Neutral	10·1	20·1	·46	·51	15·1	1·42	Absent	Absent
„ 14	940	1015	Acid	10·9	21·6	·56	·49	8·4	1·15		
„ 17	1160	1018	Alkaline	11·4	22·8	·69	·41	13·4	1·25		
„ 22	860	1024	Acid	12·3	24·8	·69	56	10·4	1·1		
Aug. 1	1300	1019	Acid	14·4	29·0	·71	·36	13·2	1·29		
„ 5	1700	1022	Acid	23·2	45·8	1·4	1·0	17	1·9		

Faeces contained ova of *Ankylostoma duodenale*.

Ocular, oral, and nasal systems normal.

Nervous system : Knee jerks normal. All superficial reflexes normal. No tremors. Pupils react to accommodation. Electrical reactions normal.

Sensations : Pain, temperature, taste, and smell normal.

Glanas : All enlarged, especially cervical and inguinal regions. Easily palpable, freely moveable, firm, not painful ; some of cervical were as large as a hazel nut.

From December 27, 1904, to August 17, 1905, the boy was under observation. He was given work in the laboratory and animal houses. No evidences of the progressing of the disease could be determined. The boy grew taller and stronger and put on weight, learnt English rapidly, and his

intelligence improved. During the winter he contracted several colds, but was otherwise well. Rheumatic pains in the knee, shoulder, and wrist joints were complained of, but quickly disappeared. Somnolence was never observed. Easily excited and naturally irritable. Outbreaks of passion occurred at times; during such an outbreak he became frenzied and incapable of controlling himself. On one occasion severe frontal and occipital pains were complained of. At the same time it was noticed that the parasites were then starting to increase in number. The temperature also rose, but after two days the trypanosomes lessened in number, and coincident with this the headache disappeared and the temperature began to fall. A trial of subcutaneous injections of arsenic having proved too painful, liquor arsenicalis was given, but he quickly complained of headache and nausea. A varied treatment of Blaud's pill with arsenic, alternating with a nux vomica tonic and then liq. fowleri alone, was given. The blood counts still further improved; the parasites became scantier, and were often absent from the peripheral blood. The temperature, which at this time was irregular and sub-normal, became more regular, though very often sub-normal. Owing to his illtemper and homesickness he was sent back to the Congo in August, and the missionary authorities were given a supply of trypanroth tabloids for him. No news[1] has been received as to the boy's progress. Lumbar puncture was performed several times, but no parasites were found in the cerebro-spinal fluid, nor was such fluid infective to animals. The fluid possessed the normal characters; it did not agglutinate trypanosomes.

Blister fluid: Serum obtained from a small blister proved negative on two occasions. Mice inoculated with it, failed to become infected. Under the chapter headed Periodicity, tables shewing the presence and absence of the parasite in the blood are given. During the last twenty-two days, the parasites were hardly ever present, and were absent for the last eleven days before he left England, apparently due to the arsenical treatment.

	Differential Count	
	March 4	June 15
Polymorphonuclear neutrophile ...	53·98	61·81
Large mononuclears	6·78	5·63
Lymphocytes	10·32	8·36
Eosinophiles	25·69	20·91
Mast cells	0·68	0 48
Transitional	2·55	2·81
	100·00	100·00

Banja. Male. *Aet.* 26.

Under observation from January 18 to April 1, 1904. Arrived in England, May 22, 1904. The early history of the case has been recorded.

Physical condition: A large, strong, very obese man. Intelligence poor. Skin soft. General enlargement of the glands. Spleen just palpable, not tender. Liver not enlarged. Urine, sp. gr. 1018, no albumen, no sugar, no bilirubin.

1. On May 18, 1905, news was received that this patient was, in the middle of April last, in the last stage of Congo sleeping sickness. 'He was not always sleeping, but greatly emaciated, talks rubbish, and seems to have lost his reason.' Further details not yet to hand, July 20, 1905.—H. E. Annett. Aug. 30, 1905, patient dead.—J.L.T.

The following analyses were made by Mr. EDIE, Laboratory for Bio-Chemistry :—

Date	Amount of Urine in c.c.	Sp. Gr.	Reaction to Litmus	Grams Total Nitrogen	Grams Urea	Grams Uric Acid	Grams NH_3	Grams NaCl	Grams P_2O_5	Albumen	Sugar
June 27	860	1014	Acid	10·5	21·3	·43	·26	3·62	...	Absent	Absent
,, 30	1440	1019	Alkaline	17·9	34·1	1·29	·6	12·2	2·5		
July 13	1500	1017	Acid	14·2	28·2	·52	·76	19·6	1·5		
,, 16	1380	1011	Acid	9·5	19·0	·51	·4	11·3	1·4		
,, 17	1480	1013	Neutral	12·6	25·3	·65	·34	10·8	1·35		
,, 22	1060	1020	Acid	10·5	21·4	·58	·34	11·1	1·2		
Aug. 1	2450	1014	Acid	20·7	41·9	1·2	·82	24·8	3·0		
,, 7	2800	1018	Acid	24·7	49·2	1·5	·97	23·1	3·4		

Nervous system : Knee jerks, cremasteric, epigastric, and anal reflexes normal. Electrical reactions normal, no tremors. Sensations, temperature, taste, and smell normal. No symptoms of drowsiness. No oedema, no skin eruption. The temperature ranged between 98° F. and 102·2° F., somewhat irregular ; sudden rises occurred which did not correspond with days on which an increased number of parasites was observed.

The pulse was as usual rapid, 94-108, once or twice rising without apparent cause to 128, usually regular and of good volume ; after climbing or work, irregular and dicrotic.

The man worked in the laboratory and animal houses. During the period of observation, from May 22 to August 15, no symptom other than the presence of parasites in the blood was noted. His appetite was inordinate and he grew extremely fat. No mental symptoms shewed themselves other than masturbation, which he had practised before arrival, and which he continued, despite all efforts to prevent it.

Lumbar puncture performed once by Dr CHRISTY, and twice while under observation, never shewed any parasites in the cerebro-spinal fluid, nor were animals infected by injections of the fluid.

Lumbar puncture was performed three times in the Congo. Once trypanosomes were found but the notes record blood being mixed with the fluid at a period when eighty parasites to a cover were present in the peripheral blood.

Gland examination : One cervical gland was removed and examined on a day when there were only a few parasites in the blood. The juice did not contain so many trypanosomes as did the blood. Glands were punctured on two occasions. On a day when the blood was negative no trypanosomes could be found in the fluid withdrawn from the gland. On the other time both gland and blood preparations contained the same number of trypanosomes.

Blister fluid : The fluid contained no parasites, but successful infection of two mice out of four inoculated is recorded after a somewhat lengthened incubation period. This case was tried with injection of sodium arseniate, but as so much pain was complained of liquor arsenicalis was given by the mouth only to be discontinued and trypanroth grs. V in tabloid form substituted. To commence with one tabloid, later two tabloids a day were employed. No inconvenience appeared to be caused. The urine became pinkish twelve days after commencing treatment. A slight reddening of the conjunctivae appeared on the eighteenth day. The saliva appeared to have a faintly pinkish hue. The trypanosomes had

decreased in numbers apparently due to the action of the dye. As the man returned to the Congo on the twenty-second day of treatment the administration of the substance had to be discontinued. The temperature had fallen somewhat and the patient appeared to have experienced no ill effects from it. The patient arrived at Leopoldville in September, 1904, and was at once put to work. Through an unfortunate misunderstanding treatment was not continued. Four months later the patient entered hospital with evident signs of 'sleeping sickness,' and died there in June, 1905.—J. L. T.

Disasi. Male. *Aet.* 26. Lower Congo.

Arrived in Liverpool, December 27, 1903. He and his wife had been found to be suffering from trypanosomiasis. His wife died on the voyage to England.

Physical examination: A well-set-up sturdy man. Muscular development good, but inclined to obesity. Intelligence good; no signs of being drowsy. Skin soft. Few chiggers in feet. General glandular enlargement.

Respiratory system: normal.

Circulatory system: Heart sounds not accentuated. Pulse, 98-101, of high tension, but regular.

Spleen and Liver: Enlarged, not tender on palpation.

Genito-urinary system normal. Urine, sp. gr. 1020. No albumen; no sugar; chlorides; no bilirubin.

Faeces contained *Ankylostoma duodenale.*

Nervous system: Knee jerks normal. Epigastric, cremasteric reflexes not increased. Pupils reacted to accommodation and light.

Ocular, nasal, and aural systems normal.

The blood at first contained no trypanosomes—but *Filaria perstans*, often twenty-six to forty to a cover. Later, trypanosomes were very scanty; the examination was often negative. On January 26 an attack of malaria occurred, the temperature reacting to quinine.

February 6: Thirty trypanosomes to a cover preparation were found; these increased to eighty to a cover, and after four days decreased to seventy; they then disappeared, to reappear again later and remain more constantly present, though in small numbers. From that time until May 7, except for slight colds and an occasional headache, nothing abnormal was remarked. Glandular enlargement had not become more pronounced. On May 8 he had coryza, and complained of pain in the right ear; this increased in intensity. Examination revealed no mastoid tenderness or discharge from the ear. Signs of pneumonia appeared on May 10, and he was removed to Dr. J. Hill Abram's Ward in the Royal Infirmary. The disease progressed, and death occurred from pneumonia, May 14.

For autopsy and morbid histology, see Part II, Disasi.

Capt. S. B. *Aet.* 25. Italian.

Patient was employed in the Congo Free State River Service. As he was suffering from trypanosomiasis and was leaving for Europe, Dr. Dutton recommended him to come to Liverpool. No definite history as to the date of infection could be ascertained: he had had several attacks of malaria. The first symptoms of irregular temperature, not reacting to quinine, and slight oedema of the legs were noticed about the end of 1901. Trypanosomes were found in his blood by Dr. Broden, in September, 1903. Patient was under the care of Dr. Lloyd Roberts in the Royal Southern Hospital from May 31 to June 18. Our notes of the case record nothing abnormal in the physical examination other than a very slight enlargement of one or two inguinal and cervical glands. The liver and spleen were slightly enlarged and palpable. No tenderness was evinced. There was no purpuric eruption, nor could oedema of any part be found while he was in Liverpool. The parasites were very scanty in the blood. The temperature was of the usual type.

On *June* 5 an epileptiform attack occurred, he became unconscious for about ten minutes, the pupils were dilated, there was slight frothing at the mouth with loud stertorous breathing.

June 6 : Another slight convulsion, the pupils were very small. These fits were the first he had had. Before admission to the hospital he had been taking tabloids of some arsenic preparation. The drug was discontinued, but as he was not doing well liq. arsenicalis fowleri was prescribed. As he wished to go to his relations in Italy he left, promising to return but never did so.

Trypanosomes were only demonstrated in the peripheral circulation on June 4 ; a monkey, rabbit, guinea-pig, four rats, and four mice were inoculated with blood on a day when parasites were present and developed the infection.

Periodicity of the Parasite in Natives[1]

Below are given the number of parasites found in fresh coverslip preparations of blood from the peripheral circulation. These columns read downwards show the periodicity of the parasite.

Mpangila			Disasi			Banja	
3	1	4	0	0	0	46	10
2	4	1	0	30	0	32	0
1	2	0	0	50	0	14	0
0	4	0	0	70	0	300	0
2	3	3	0	0	0	4	0
1	6	2	0	0	0	16	1
0	1	5	6	2	2	40	0
0	2	2	0	0	2	40	c
1	0	1	2	4	0	0	0
0	3	2	0	2	0	0	0
2	6	7	0	0	0	8	0
0	8	5	0	2	0	2	0
0	6	2	0	2	0	0	0
0	0		0	0		16	0
0	2		0	0		16	0
1	2		0	4		4	0
0	4		0	2		0	0
1	0		0	0		0	0
0	0		0	0		12	0
0	2		0	0		0	1
1	2		0	2		0	90
0	0		0	0		0	21
0	0		0	2		0	2
5	2		0	0		48	0
0	6		0	0		6	0
0	8	rise of temp.	0	0		0	5
0	34	100·6	0	0		0	11
0	54	103·4	0	4		0	
0	12	101·4	0	0		3	
4	8		0	0		0	
5	2		0	0		1	
0	6		0	0		0	
0	14		0	0		7	

1. In a later report, periodicity of the parasite in animals as compared with other trypanosomes will be discussed.

PERIODICITY OF THE PARASITE IN NATIVES—*Continued*

	Mpangila	Disasi	Banja
Days present	113	22	26
Days absent	104	78	33
Longest period present—days ...	12	3	8
Longest period absent—days ...	7	25	13

These figures show that periodicity is very marked in some cases. The charts of these cases do not record any very marked rise of temperature coincident with an increase in the number of parasites. Mpangila had the parasites more constantly present but in smaller numbers than in the other two cases. From the periodicity columns the case of Disasi shows well the difficulty in making a diagnosis without daily examinations continued for a long period.

II. INOCULATION EXPERIMENTS WITH *TRYPANOSOMA GAMBIENSE*

Strains of Trypanosomes Derived from Man

In May, 1904, a preliminary note was published on a comparison of the animal reactions of various strains of trypanosomes derived from sleeping sickness cases in Uganda and the Congo Free State, strains from native fever cases from the same regions, with the original strains of trypanosomes brought back by Dutton and Todd from the Senegambia. Two Gambian strains were derived from natives suffering from the so-called 'Trypanosome or Native Fever'; the third was from a European who had died from trypanosomiasis. The strain obtained from the boy at Gunjur has been the principal Gambian strain employed, though the other two have been used to some extent. It is the 'Gunjur' strain which was sent to Professor Laveran, and also to Colonel David Bruce; from the latter we obtained Uganda sleeping sickness strain and Uganda native fever strain. From our expedition in the Congo Free State we have secured a large number of strains. We have been able to obtain fresh strains of trypanosomes from two cases of sleeping sickness and three native fever cases which had been sent from the Congo Free State to Liverpool for observation and treatment. We have also been able to procure a strain from a European who had returned from the Congo suffering from trypanosomiasis. It has been of great assistance to us to be able to compare strains derived from so many sources, and especially to be able to procure strains in their first passage through an animal and to compare them with those strains which had been through several hundreds of passages, and finally to compare them all with the parasite in the blood or cerebro-spinal fluid of a case suffering from the disease. In our report in the *Lancet*[1] we gave in detail the results of the inoculation of many of these different strains. Dutton and Todd have already published their results with the Gambian strains, also a short account of the animal reactions with various strains from the Congo Free State. Laveran and Mesnil have published their findings. Drs. Brumpt and Wurtz have recorded the results from strains derived from French Congo cases. All groups of observers have come to the conclusion that the trypanosomes found in cases of sleeping sickness are identical with the trypanosome found in the blood of a European, and described by Dutton in 1902. It would, therefore, seem unnecessary to reiterate any of the findings were it not for the publication by Plimmer of a comparison of the Gambian fever strain (Gunjur) and the Uganda sleeping sickness strain,

1. *Lancet*, May 14, 1904, p. 1337.

both obtained by him from Col. BRUCE. A reply to the publication by PLIMMER has already been made.[1] In correspondence with Professor LAVERAN, who has also experimented with both of the strains used by Mr. PLIMMER, he writes that his results are equally opposed to PLIMMER's. In order that Mr. PLIMMER's findings may be compared with our results, a synopsis of his report is printed.

Further experiments and comparisons have been made with the various strains of trypanosomes. We have been able to successfully inoculate four baboons, one *Cynocephalus sphinx*, three *Cynocephalus babuin*, with strains of *T. gambiense*; these animals have all died. The baboon is certainly the most resistant animal with which we have experimented. From our baboon experiment, No. 747, a highly virulent strain has been recovered by passage through a rabbit.

BABOONS (*Cynocephalus babuin*)

Experiment No. 709. Inoculated intraperitoneally July 24, from Rhesus, 672. Mixture contained two to six trypanosomes to a field. The monkey, 672, was a direct inoculation. On August 10 a temperature of 104·6° F. was registered; no parasites were seen. The animal lost weight, and the blood counts showed a diminished number of red corpuscles and of haemoglobin. No parasites were seen up to death, October 7. During the ten days preceding death the animal became very emaciated, and for the last thirty-six to forty-eight hours weakness was marked. Baboon lying down and almost unconscious. A rabbit inoculated with nearly ten c.c. of heart blood after a greatly prolonged incubation became infected. Up to date it has never shown parasites in large numbers.

Experiment No. 747. Inoculated intraperitoneally August 21, the temperature rose on the sixteenth day and after that date continued very irregular—104·2° F. being frequently registered. Death occurred on October 10, being hastened by a severe dysentery which commenced five days before. Parasites were first seen on October 1 and again on October 4, each occasion a high temperature was registered. In this case, as also in the other two, though the blood was so often or always negative still the characteristic clumping or autoagglutination[2] of the corpuscles was pronounced. This phenomenon occurred gradually but was easily determinable about the eleventh to fourteenth day after inoculation; it persisted to the end. At the autopsy, which was done immediately after death, a rabbit, 823, and two guinea-pigs were inoculated with large amounts of blood which was negative even when centrifuged. The rabbit littered on October 25, at the same time the temperature rose, the autoagglutination of the corpuscles was present but no parasites were seen. On November 1, 105·1° F. was registered; the next day, the twenty-third after inoculation, parasites were seen; these increased very rapidly so that thirty to forty to a field were present. From this rabbit

1. *Proc. Roy. Soc.*, 1905. 2. Dutton and Todd.

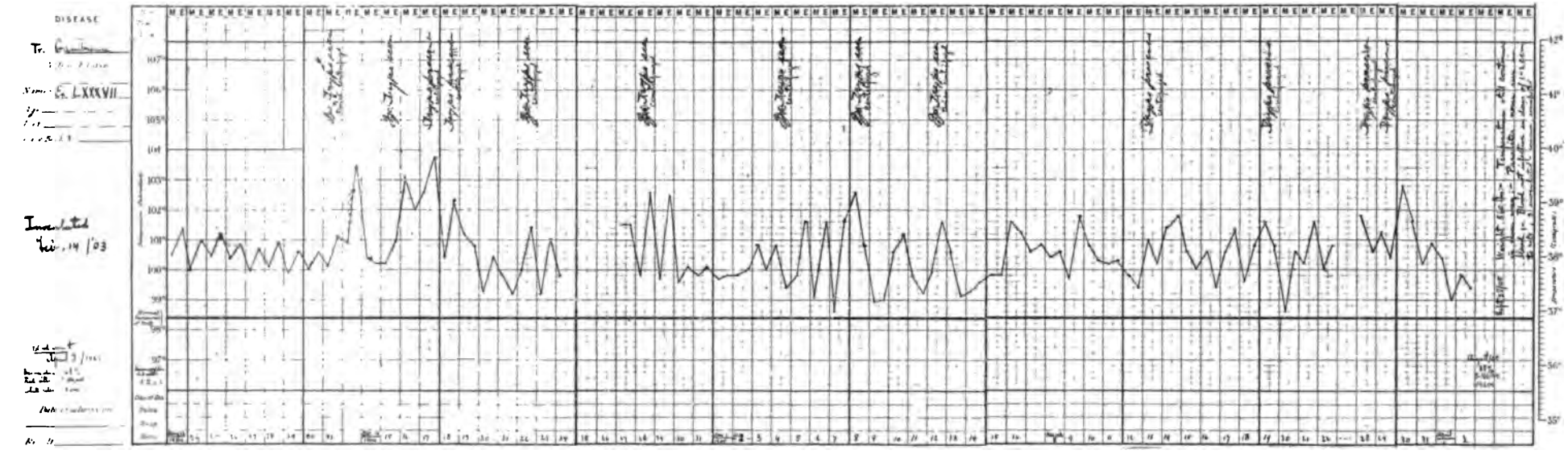
a series of animals have been inoculated. The incubation period and duration of the disease is much less than with the ordinary strain of parasite. One of the guinea-pigs has since shown parasites in its blood. Whether the parasite from this guinea-pig is as virulent as that from the rabbit is not as yet determined. The animals died four-and-a-quarter weeks after the appearance of parasites, there being over one hundred to a field at death. Further experiments in progress.

Experiment No. 890. Inoculated intraperitoneally with 9·0 c.c. pure blood from rabbit 877. Mixture contained one parasite to a field. This rabbit was inoculated in the third passage from rabbit 823 (see above). On the tenth day the temperature rose to 103·4° F. No parasites were seen. Two days later a trypanosome was discovered. From that time until the baboon's death on the forty-first day the organisms were present at nearly every examination. Death took place suddenly. A pup inoculated with heart blood from this baboon developed the disease after a prolonged incubation, the parasites were present in only very small numbers up to its death.

All three baboons showed a rise of temperature to 103°-104° F., and following on this an irregular temperature which persisted, usually becoming lower and often being sub-normal before death. Parasites were found in the blood of only two of the baboons despite repeated centrifuging of the blood, but though the parasites were hardly ever present, and then only one to two to a cover, still animals inoculated with large amounts of their blood have developed the disease. Loss of weight and anaemia, though gradual, was present in all three cases. The autopsies, especially of 747 and 709, showed an enlarged and firm spleen, slight enlargement of the lymphatic glands. Dr. BREINL has included the brains, cords, and organs of these animals in his report on the histo-pathology.

The strain derived from baboon 747 and passed through rabbit 823 has been inoculated into a great number of animals. In all the incubation period has been greatly shortened. Rabbits very often show parasites in their blood in two to four-and-a-half days, and death may occur as early as the fifth to eleventh days after inoculation. In many cases the parasites augment from day to day until there will be one hundred and fifty to two hundred or more to a field. The animal will very often suddenly die. The number of trypanosomes will frequently decrease, they usually commence to increase in numbers again in three to eight days, and continue to do so until the blood actually swarms. A very severe anaemia occurs, loss of weight is marked. Coma may develop a few hours before death or the animal may die while in the act of eating and apparently quite strong. The temperature is usually very high, the incubation rise being often to 104°-106° F. It usually continues high, hardly falling at all in the acute fatal cases. The *post-mortem* of these acute cases shows a multitude of parasites in every organ and in the blood and serous exudates, acute swelling of the spleen, enlargement of the glands—some of these may be haemorrhagic.

CHART IV



to face p 15

Rats, mice, dogs, cats, guinea-pigs, and monkeys develop the disease in equally short incubation periods. Death occurs early: in the dog in nine days; cats, twenty days; rats, twelve to eighteen days; mice, twelve to sixteen days; monkeys, ten to twenty-four days. Guinea-pigs may die in twelve days to three weeks after the appearance of the parasites in the peripheral blood. The guinea-pig may resist, and after an incubation of three to five weeks acquire a more chronic form of the disease.

Monkeys

Various species have been used, *Macacus rhesus*, *Cercopithecus callithricus*, *Cercopithecus ruber*, *Pithecia satanas* (*Jew* and *Sooty* monkeys). All react to inoculation with almost the same sensitiveness. The *Sooty* appears to be somewhat less so, the parasites are rarely seen but autoagglutination of the corpuscles, anaemia, fever, and loss of weight occur as in the others. Monkeys, though reacting so well and the parasites often showing in large numbers, are, unfortunately, extremely liable to digestive disturbances, their vitality is not great and they quickly succumb to the complication. As they usually harbour large numbers of intestinal parasites the blood counts cannot be absolutely depended on. The monkeys infected with sleeping sickness strains have not shown any more symptoms of 'sleep' than others inoculated with 'Gunjur,' etc., strains. Monkeys infected with *T. dimorphon* and non-infected ones dying from dysentery, etc., have sometimes exhibited even more pronounced symptoms.

It is of interest to record the progress of the disease in large animals infected for a long time.

Horse VII

Experiment No. 87. Inoculated by Dutton and Todd, February 14, 1903. Strain 'Mr. Q' (European, Senegambia). Parasites appeared March 16. Up to May, when it was sent to England, the organisms were fairly constant in the peripheral blood. From August 31, 1903, the blood was negative to microscopical examination, but still infective if inoculated in large amounts. On numerous occasions, the blood was centrifuged, but no parasites could be found. The blood when inoculated in amounts of 0·5 c.c. into rats and mice, formerly proved infective, such a quantity is at present of no use; 2·5 to 3·0 c.c. or more is now needed to produce an infection. Moreover, it is necessary to use more than one animal, as the animal may only acquire a mild chronic infection with very few parasites to be seen in its blood. The horse, shortly after the infection, lost flesh but improved after arrival in England. It is now in excellent condition, weight, 510 pounds, no oedema. The blood count: reds, 5,600,000; whites, 17,600; haemoglobin, ninety per cent. Urine normal. The autoagglutination of the blood corpuscles is still present. Its temperature has been recorded for nearly the whole period (see chart); glandular enlargement has not been noticed since the animal arrived in England.

Donkeys

Two were inoculated, one with 'Gunjur' strain (*Lancet*, May 14, 1904), the other with Uganda sleeping sickness strain, between February and March, 1904. These animals became infected, and showed the parasites in their blood. The parasites have always been extremely scanty in numbers, even after centrifuging the blood. The occasions on which the blood examination has been negative have gradually become more frequent, but they are still occasionally seen. The autoagglutination of the corpuscles is marked. Few symptoms have been observed. For about two months after infection, the animals appeared to be losing flesh and were less lively, they have now regained their normal weight. Anaemia has been present from the commencement but it is not marked. The temperature has continued irregular, varying between 98·6° and 100·4° F., occasionally it rises to 101·8° to 102·4° F. about every ten to fourteen days or so. This rise continues for about forty-eight hours, when the temperature drops to normal or sub-normal. The urine is negative. Glandular enlargement is present in both these animals, it was noticed about a month after infection, and still continues, though much less marked. The blood is infective to rats in amounts of 0·5 to 1·0 c.c., formerly a few drops of blood were sufficient.

Cow

This animal was inoculated, April 28, 1904, the temperature rose to 103·6° F. eight days later, but no parasites were seen until May 17. A rat inoculated on the day of the rise, developed the disease. For the first month no symptoms were noted, the animal then began to lose flesh, and became quite thin, but in July it commenced to put on weight. The yield of milk decreased, the parasites were extremely scanty, and have been present in less numbers and at longer intervals than in the case of the donkeys and the horse. Anaemia was not marked. The blood count at present is, reds, 6,220,000; whites, 16,200; haemoglobin, eighty-five per cent. Autoagglutination of the corpuscles has been present from May 20—it still persists, but is not so marked. The temperature has shown the same irregularity, rising gradually but irregularly for ten days to remain high for about one-and-a-half to three days, and then commencing to fall. Glandular enlargement was present up to August, 1904. The coat which had become rough and dull looking is now smooth and glossy. The blood is still infective though larger amounts are now necessary.

A peculiar condition has been noted in the milk. About two-and-a-quarter months after infection, it was noticed that the milk was very poor in fat, and watery. Dogs fed with it developed in about twenty-four to thirty-six hours a very profuse diarrhoea, which continued so long as the milk was given. Pasteurising or autoclaving at 120° C. appeared to make no difference, as pups and kittens developed diarrhoea in two to four days. Bacteriologically, the milk has been proved to be free from pyogenic organisms. Guinea-pigs inoculated with it have not developed any lesions.

Chemical analyses have failed to detect any changes other than a low percentage of fat. Tried on human beings, nothing is noted other than a tendency to cause biliousness and diarrhoea. The possibility of antibodies being formed in the milk has been recognised, but no proof has been established.

Sheep

A wether, Experiment No. 560, became infected ten days after inoculation. On the twenty-seventh day one parasite to a field was noted. The numbers gradually decreased, and five months later, the animal appeared to have recovered. Blood in amounts of ten to forty c.c. was non-infective. Slight anaemia was present for a time. Autoagglutination was fairly well marked until the end of the third month.

Goats

The animals inoculated with the various strains have all died. One, No. 480, inoculated with Uganda sleeping sickness died on the fifty-eighth day. The anaemia was somewhat marked. The parasites were present fairly constantly, usually four to twenty-four to a cover preparation being seen. Autoagglutination of the corpuscles was not very marked. The temperature remained irregular. Loss of weight was the only symptom noted. The *post-mortem*, twelve hours after death, showed a slightly enlarged spleen and glands, none of them haemorrhagic. Decomposition was advanced.

Dogs

These animals, though easily susceptible to inoculation, survive for many months. Laveran records one dog still living, six months after infection. A bitch, No. 738, inoculated with 'Gunjur' strain lived for over nine months. The parasites were at first hard to find, but for the last three months became more and more numerous. For the first two months emaciation was not marked; a progressive decline then became evident, and this continued up to death. Towards the end a profuse discharge from the eyes occurred; the conjunctivae were inflamed and infiltrated.

The blood count of this animal is interesting (p. 18).

Some dogs have died in three weeks, others have lived for varying periods up to nine months. A bitch, Exp. 45, brought back by Dutton and Todd, recovered from the disease. This animal had been inoculated when a puppy; it developed a chronic form of the disease and at the end of four months no parasites could be found in its blood. Autoagglutination of the corpuscles gradually became less marked, and ten months after inoculation the blood appeared perfectly normal. In amounts of 2·0 to 10·0 c.c. it was non-infective. Reinoculation of small quantities of blood failed to produce infection, although a control dog developed the disease.

EXPERIMENT 738

Day of Disease	Red Corpuscles	Leucocytes	Haemo-globin	Parasites
4th	5,240,000	12,000	85	8 to cover
5th	6,500,000	12,600	86	40
6th	5,43c,000	15,800	75	228
7th	5,610,000	17,000	65	400
8th	5,850,000	18,600	64	2400
9th	5,540,000	13,600	79	24
15th	4,270,000	18,400	60	56
58th	5,260,000	10,000	69	28
69th	7,820,000	11,800	92	2
166th	4,310,000	14,000	70	680

It appeared to be immune. Its serum did not possess protective properties. Infection and progress of the disease occurred in all animals treated with this serum. Its agglutinative properties were infinitesimal. Pups born of this bitch were as susceptible to the various strains as the control ones. The temperature varies. Usually, in the long continued cases, exacerbations of fever with apyrexial periods of eight to ten days occur ; sometimes the fever is high and irregular during the whole course of the disease. A subnormal temperature is usually recorded before death. One animal developed fits eight days before death, and unconsciousness became marked eighteen hours before the end. Owing to absence from the laboratory no *post-mortem* was done. Anæmia may or may not be pronounced. Autoagglutination is usually pronounced.

Pups are very susceptible and usually show large numbers of parasites in their blood. Periodicity is sometimes very pronounced, the parasites often diminishing from ten to forty to a field to only a few in a coverslip preparation. This may occur in the course of a few hours.

CATS

As shown by us, these animals are susceptible to inoculation. The disease runs a course of three to ten weeks ; a few have lived for a longer period, six-and-a-quarter months. Parasites are generally hard to find, but can be usually found if properly looked for. The temperature is irregular, sometimes passing 104·5 F., apyrexial intervals usually occur. It is often subnormal about twenty-four hours before death and there may be a steady and regular fall for some days preceding death.

Anaemia may be a prominent feature. The blood count of one cat was, reds, 8,600,000; whites, 6,000; haemoglobin, seventy-eight per cent.; on the twenty-ninth day of infection, reds, 3,470,000; whites, 14,400; haemoglobin, thirty-eight per cent. Its weight had decreased three hundred and twenty grammes during this time. The parasites in this animal were numerous, at times three to a field were seen. Auto-agglutination is a noticeable feature both in the acute and the chronic types of the disease.

Kittens are very susceptible, the parasites appearing early and generally persisting in large numbers until death. The anaemia is severe. Loss of weight and stoppage of growth occurs. A high temperature is noticed. The duration of the disease is from three to seven weeks. Purulent conjunctivitis is common.

Rabbits

The incubation period has not varied markedly; from the figures given in the preliminary report five to fifteen days. Some of the rabbits have lived one hundred and fifty to two hundred and seventy-three days; these have had a chronic, mild infection. Average duration fifty to one hundred and twenty-eight days.

Guinea-Pigs

The disease varies, many of the animals have only shown the parasites after one to three months or more. The trypanosomes have then continued present in large numbers, death occurring fourth, ninth, and sixteenth weeks after the appearance of the parasites in numbers. Rupture of the spleen has caused death in four cases, in every instance the blood was full of trypanosomes and the spleen acutely swollen.

Morphology of the Parasite

Dutton and Todd, Laveran and Mesnil, Bruce, and others have described the parasite. Both Dutton and Todd, Laveran and Mesnil, and Bruce have had opportunity of comparing the trypanosomes found in the blood with those parasites found in the cerebro-spinal fluid and those present in the blood of inoculated animals. All groups of observers have agreed that they can determine no marked differences. All agree that the parasites in the cerebro-spinal fluid are often vacuolated. As Laveran and Mesnil point out and illustrate in their work (Fig. XLII, Nos. 1, 2), and Dutton and Todd (Pl. I, Fig. 1), the trypanosome may contain large vacuoles. Laveran and Mesnil ascribe it to the parasite being poorly fixed, and show that with very serous blood such a condition is common. Such being the case it is only natural that films made from the cerebro-spinal fluid will contain many of these vacuolated forms.

We have compared the trypanosomes in the same way as these observers and can find no difference between the parasite present in the cerebro-spinal fluid and blood of patients,

or in the blood and exudates of infected animals. PLIMMER claims that the parasite of 'Gambia Fever' differs from the sleeping sickness trypanosoma: the former being longer, generally larger, and more easily stained than the stumpy, larger-vacuoled, badly-staining trypanosoma of sleeping sickness. After the examination of many animals infected with the various strains of *T. gambiense* the distinctions which PLIMMER notes appear to be artificial. Both long and short, large and small forms are seen in their blood; on comparing these with the parasite in the blood of a native the same forms are seen. No differences have been noticed in the staining reactions. The parasites in serous blood require longer staining than do those in less anaemic blood.

Comparisons were made by injecting animals both with a sleeping sickness and a 'Gambian Fever' strain. It was impossible to detect any difference whatsoever either in the control films or films made from the animal. Animals were injected with blood containing a majority of short stumpy-formed parasites, these animals have shown as many or more longer forms than stumpy ones in their blood. Careful measurements have not shown any difference between the sleeping sickness or 'Gambian Fever' or any other 'Trypanosome Fever' parasite. These comparison slides were made from the same species of animal, and, with two exceptions, the number of passages of the parasite was the same. Chromatic granulation is common both to the long and short forms. Parasites appear to possess these granules more often if the infected animal is dying or if large numbers of parasites are present in the blood. They have also been prominent in the trypanosomes undergoing degeneration in the blood of an animal treated with arsenic or trypanroth. In some cases they have almost filled up the whole body of the trypanosome, making it difficult to distinguish the micronucleus and interfering with the macronucleus. These granules may form in clusters or may be ranged in comparatively regular rows, they may vary from a pin-point to a size equal to or larger than the macronucleus in size. Trypanosomes containing such granules may be run through several passages and still possess granules. One mouse, Experiment 692, had enormous numbers of trypanosomes containing these granules; all the subinoculated animals became quickly infected, and though this strain was run through eleven passages the parasites still contained the granulations. The granules were present in both single and divisional forms of the micro-organism. These granules were present in the trypanosomes of the subinoculated animals—rabbits, rats, mice, and pup. In a monkey a less number of granules were seen. The twelfth passage into a guinea-pig infected the animal, but the trypanosomes had almost lost the granules. In the blood of this animal granular trypanosomes were noted from time to time; it died from broncho-pneumonia; its subinoculated rats have never shown granular forms of the parasite.

Chronicity

The artificially produced disease in animals may pursue an acute or chronic course. With the ordinary strain all the animals show a more or less chronic and lengthened infection as compared with Nagana and other pathogenic trypanosome infections. It appears more virulent for kittens, pups, and monkeys (*Rhesus*) than for cats, dogs, rabbits, guinea-pigs, rats, and mice; in some of these animals the parasites, after appearing almost constantly for a time, disappear and are then found only at rare intervals. Such animals usually show an increase of parasites a short time before death. The large animals, goats, sheep, cows, donkeys, and horses, though susceptible to the infection, with the exception of goats, appear to be able to withstand the disease. It is interesting to note that a horse still shows infection twenty-seven months after inoculation.

Virulence

In the majority of animals infected with the various strains of *T. gambiense* the disease pursues a normal course, and the animal dies with a fair number of parasites in its blood. Occasionally the numbers of parasites increase enormously, and such an animal quickly dies. The subinoculations, even with very diluted blood containing no more parasites than control blood, may show that it is extremely virulent, the incubation and duration of the disease being shortened. Such virulency has been noted in baboon 747, rabbit 823, and in rabbits, rats, and mice. Though parasites are numerous in guinea-pigs, in no case has an increased virulency of the parasite been observed. As noted elsewhere if a strain be repeatedly and quickly run through animals of the same species it will acquire a certain virulency for such a species, but this continues only so long as the strain is not run through other species of animals.

Immunity

Certain species of animals appear to possess a more or less stable resistance to infection with trypanosomes. Baboons are peculiarly hard to infect, the immunity is not an absolute one as they have been on occasion infected with *T. gambiense* and *T. dimorphon*. In every case the incubation has been lengthened, the parasites have been scanty but the animals have succumbed to the disease. Certain species of monkeys, as the *Jew* and *Sooty*, are somewhat more difficult to infect than the *Rhesus* and *Calothrix*.

All attempts to infect birds and fish with *T. gambiense* and *T. dimorphon* have failed. Goats[1] and sheep, though susceptible to infection with various pathogenic trypanosomes, do not easily succumb. Some species of animals, ordinarily susceptible

1. As Bruce suggested it is with such animals that the histo-pathology of the disease ought to be investigated. In our report a comparison of the lesions found in animals dying after lengthy period from a chronic type of the Gambian Horse disease has been made with those found in sleeping sickness. A comparative study of a large number of goats, etc., infected would be of great aid in determining if the various trypanosomes pathogenic for animals produce lesions akin to those found in natives dying from sleeping sickness.

to infection, show occasional resistance. One rat, 285 (see p. 25), was difficult to infect with *T. dimorphon*, and then only showed the parasites for a few days. It recovered and was able to withstand inoculations of enormous quantities of virulent blood. In the preliminary note on *T. gambiense* it was shown that sometimes, of two rats inoculated at the same time the one acquired the disease the other did not, the resistant animal even having received the greater amount of the virulent blood.

With these resistant animals and those in which the disease pursues a markedly chronic course experiments have been made to see if an exacerbation of the disease could be produced. Such animals have been daily, weekly, or monthly reinoculated with small quantities of virulent blood. In a few cases a rise of temperature corresponding to the incubation period has been observed. In a couple of cases the parasites have somewhat increased. Various experiments in breeding infected and healthy animals together have been done. The progeny of such unions have not shown any immunity or resistance.

Endeavours to protect animals, or to at least mitigate the disease by injection of attenuated parasites or large quantities of blood from which the parasites have been freed or killed off, have proved of little value. The same applies to injection of animals with sera from animals which have recovered from the disease in which the chronic type prevails, or from animals naturally immune. Here again very meagre results have been obtained. Efforts to produce an immunity by the administration of drugs is of little value.[1] Arsenic, which has an effect on the parasite, is of no use when attempts are made by first treating the animal with the substance to render it later proof against inoculation of virulent blood. EHRLICH and SHIGA in their work on 'Trypanroth' were able to protect' animals by preliminary treatment with the dye. If large enough amounts are administered a certain amount of protection is afforded.

TOXICITY

All efforts to isolate a toxine have proved negative. The various procedures which PLIMMER and BRADFORD had used were employed. They had found that animals could be inoculated with large quantities of blood from dogs infected with Nagana, the blood being filtered through porcelain bougies, without producing any effect. As much as 150 c.c. of filtered serum have been used without producing any toxic effect.

On one or two occasions the injection of blood containing trypanosomes has seemed to us to possess toxic properties. A rabbit infected with *T. gambiense* showed this. The blood as long as there were few parasites present could be inoculated into rats with impunity. Later on, as the parasites became numerous, it was noticed

1. Such a line of investigation with this and other substances ought to be tried on large animals. The history of punitive expeditions, etc., in the past has shown how handicapped man is when a trypanosomic disease breaks out amongst the transport animals. If this dye were administered before passing through a fly-belt, and the animals carefully examined and tested before and afterwards, valuable data would be available.

when the blood was inoculated that rats which received 0·5 to 0·8 c.c. of pure blood died in twenty to twenty-eight hours, while controls injected with a weaker solution, 0·1 to 0·5 c.c., survived. On three consecutive days batches of four rats were inoculated intraperitoneally ; the two animals which received the higher amounts were killed, the other two survived. A repetition of these experiments gave the same results. Cultures made from the rats remained negative, and films from the inoculation mixture, peritoneal cavity and heart blood of the rats, showed no bacteria. This rabbit died a week later. Maceration of its organs and treatment in the usual manner failed to reveal a toxine. The organs of animals dying or killed when numerous trypanosomes were present in the blood and in the juices of the organs, or at any time when the parasites have almost disappeared, have been used. The precipitates obtained from them have neither produced any effect nor afforded any protection against infection. The organs of the two cases of sleeping sickness were tried with like negative results. Peritoneal and pericardial exudates and fluid from oedematous tissues containing parasites have been ground up and injected also with negative results. It is possible, if they could be procured in large quantities, that some substances might be obtained which would cause a reaction, but the quantity of fluid ordinarily obtainable is limited. Centrifugalization of large quantities of blood renders it possible to collect enormous numbers of trypanosomes, but they are mixed with leucocytes. Efforts to get rid of these latter have not been successful. Inoculation of these parasites after they are killed by heat or cold or kept for a sufficient time have failed to produce any effect. From the products of culture tubes no toxine has been found, and animals inoculated with such contents have shown no evidences of intoxication.

Agglutination

The possibility of the agglutination of the parasite acting as an adjunct to the diagnosis of the disease has been realised. The results are not satisfactory. Blood, from an animal showing parasites, when defibrinated and mixed with serum from a native or animal suffering from the disease has been used. The parasites do not agglutinate. Serum from an animal continually injected with living trypanosomes shows the same negative results. In both cases there is a certain agglomeration of the parasites, but this is also usually obtained if normal serum be used. The serum from a native or animal added to defibrinated blood from the same case shows a slight agglutination. The exudate from a bleb causes no definite agglutination. If the trypanosomes be rendered immobile by the addition of weak formol before the serum is added, a partial clumping of the parasites may occur. Brumpt and Wurtz added potassium citrate solution to blood containing parasites to prevent coagulation, and then added serum from a sleeping sickness case. A rapid agglutination of the trypanosomes occurred. As they point out citrated blood containing parasites shows agglutination of the trypanosomes in a short while. In our opinion blood, to which

citrate is added, must of necessity be of no value for agglutination diagnosis on account of spontaneous agglutination of the parasites. With defibrinated blood the objection is that the agglutination of the blood corpuscles seen so often in animals or man suffering from trypanosomiasis renders it impossible to say whether a true agglutination of the parasites is produced.

Serum from the horse, cow, donkey, sheep, or goat, if added in large quantities, produce an agglutination; it is perhaps a trifle more marked if the animals have been infected[1] or cured. Baboon serum agglutinates the parasites to a certain extent, the action seems to increase and remain, as far better agglutinations are observed some hours after the commencement of the experiment.

Conclusions

1. The further comparison of the trypanosomes found in (*a*) the cerebro-spinal fluid of Uganda sleeping sickness cases; (*b*) the cerebro-spinal fluid and blood of the Congo Free State sleeping sickness cases; (*c*) the blood of Congo Free State 'Trypanosome Fever' cases; and (*d*) the blood of Europeans infected in the Congo confirms the previous observations that all these trypanosomes are identical in animal reactions and morphology with *Trypanosoma gambiense* (Dutton).

2. There is no acquired immunity against infection nor transmission of immunity to offspring.

3. That baboons, *Cynocephalus babuin* and *Cynocephalus sphinx*, are susceptible to infection with *T. gambiense*. That usually a chronic type of the infection develops but a fatal termination occurs.

4. That in many of the animals, especially the horse, cow, donkey, sheep, and goat, the infection is of a mild character, but their blood is still infective to susceptible animals one year after the infection. That in the case of the horse the parasites are still occasionally seen and the blood infective twenty-eight months after inoculation.

5. That periodicity of the parasite is a prominent feature both in man and beast.

6. That the passing of a strain from a susceptible into a very resistant animal does not attenuate the organism.

7. That the parasites in an animal may sometimes become more virulent. The numbers increasing enormously, the subinoculated animals become more rapidly infected and death occurs. That such a strain may be particularly virulent for one species of animal. That the more rapid infection is not due to the inoculation of a greater number of parasites than usual.

8. That the parasites, after being passed through many hundreds of animals for nearly three years, still retain its morphological characters, and animals inoculated with it react as described by Dutton and Todd.[2]

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1. The sera from animals infected with other trypanosomata usually cause almost as much.
2. Liverpool School of Tropical Medicine, Memoir XI.

III. *TRYPANOSOMA DIMORPHON.* GAMBIAN HORSE DISEASE

This strain was brought back by Drs. DUTTON and TODD from the Senegambia. Our results in general coincide with those of DUTTON and TODD and LAVERAN and MESNIL.

RATS—WHITE AND BLACK

The incubation varies; in the case of the more attenuated strains the incubation period may be as long as twenty days; with the ordinary strain, which has been passed through many rats, the incubation period is from three to twelve days, the average being from four to seven days. Duration of infection, seven to forty-two days; average, eighteen days. Periodicity in some of the rats is fairly well marked. A white rat in the course of thirty-three days showed almost daily an irregular number of trypanosomes in the blood—after amounting to thirty to forty to a field they would diminish to one to thirty to forty fields, and then gradually rise. Each time when the parasites commenced to disappear a marked leucocytosis was present. On account of the smallness of the animal no blood count could be made.

One rat showed a marked resistance to the parasite:—Experiment 285, rat (white), one hundred and eighty-five grammes weight. Inoculated intraperitoneally January 5, 1904, with 0·75 c.c. of blood containing three trypanosomes to a field. Up to February 5, this animal never showed parasites in its blood. It was then reinoculated intraperitoneally with the whole blood from a rat (Experiment 242), there being two parasites to a field. Five days later four parasites were seen to the cover-slip preparation. These continued present for three days; its blood then became negative. On February 22, nine days later, it was once more inoculated intraperitoneally with 3·5 c.c. of pure blood from a dog (Experiment 314), there being twelve trypanosomes to a field in the mixture. It never became infected, nor was its blood infective to small rats when inoculated in quantities of 0·5 c.c. to 0·75 c.c. If some of its serum was added to blood containing the parasites a marked and permanent agglutination occurred. Up to May 10 it was repeatedly inoculated with large quantities of virulent blood, it then succumbed to an epidemic of bronchopneumonia. Unfortunately, very little serum could be obtained for agglutination and other work. Artificially a certain degree of resistance to this parasite can often be established by injecting a mixture of blood and citrate solution attenuated by preserving in the incubator at 35° C. for four to six hours. Out of seven white rats so treated five of them were able to withstand at the end of eighteen days inoculations of 1·0 to 3·0 c.c. of virulent blood. Their blood, however, caused very slight

agglutination. If these animals were bled very freely, a new inoculation of virulent blood would cause them to become infected, and after that the disease pursued the normal course. The spleens of rat 285 and some of the five others not showing the infection were small and normal looking, the glands were not enlarged. The spleens of rats dying after a duration of more than two weeks are very markedly enlarged, as pointed out by LAVERAN and MESNIL. The glands are also enlarged and many, especially the retroperitoneal group, are haemorrhagic. The pleural surface of the lungs is very often spotted with small petechial haemorrhages. Oedema has only been noticed on two occasions. Paralysis has never been noted in rats dying from the acute form. Two rats suffering from the chronic form developed a partial paresis of the left-hind leg. Haemorrhagic nephritis has been noted in two large rats suffering from the chronic type, the urine contained a few trypanosomes, the kidneys showing numerous small haemorrhages on their surface and in their substance.

MICE—WHITE, BLACK, AND GREY

The incubation varies from two to five days. Death may occur in the acute cases within sixteen to twenty-three days. In the more chronic cases the duration may be from thirty-seven to one hundred and thirty days. In the acute cases the parasites appear and continue to augment almost up to death. In the chronic phase of the disease there is very often a marked periodicity, the parasites being absent from the blood for intervals of eight to fourteen days. Subinoculations at such a time into young rats or mice are negative if small amounts of blood are used.

Two mice inoculated intraperitoneally with blood from Horse VI (p. 30) in amounts of 1·5 to 2·0 c.c., the blood being negative to microscopic examination, never showed parasites until the fourteenth and fifteenth days, the parasites then began to increase and the disease terminated eight days after the appearance of trypanosomes. Two mice were inoculated from the horse at the same time and never became infected, but were later on shown to be immune.

As LAVERAN and MESNIL note, the spleens of mice infected may become enormously enlarged. The organ often being six times as large as normal, causes a most noticeable tumour and almost completely filling the abdominal cavity compresses the other organs. In mice treated with arsenic the splenic tumour has lessened at the same time as the parasites have temporarily disappeared from the blood.

GUINEA-PIGS

Over twenty-five have been used. The incubation has varied between four to fifteen days, the average being four to six days. The duration of the disease is from nine to sixty days. The acute cases showing parasites in large numbers in the blood very often die in thirteen to twenty days. Young guinea-pigs succumb sooner than do adults. There is usually a slight rise of temperature on the appearance of

parasites ; after which the temperature may vary very little from the normal. Loss of weight is usually a feature of the disease. Anaemia is present but is not so marked as in rabbits infected with *T. dimorphon*. Paralysis of the posterior limbs has been seen in two cases about thirty hours before death. The parasites after the first appearance usually continue constantly in the peripheral blood, gradually increasing until there may be forty to sixty to a field. These high numbers may continue for ten days before the animal dies, or diminution in the number coincident with an increase in leucocytes can be noted.

Periodicity in some cases is a prominent feature, the parasites at times almost completely disappearing and remaining so for some days. This feature is observable in the more chronic cases. Rupture of the spleen has occurred in seven cases. Five of these cases were of the very acute form in which, after the incubation period, a continuous and rapid increase of the parasites had been noted. In the remaining two cases the disease was more prolonged. One death happened on the ninth day of the disease ; numerous parasites were present in the blood, and the animal died suddenly. The abdomen contained a large quantity of fluid blood, no lesion of the spleen or other organ could be detected, but the peritoneum was studded with small pin-head sized haemorrhages. One case of haemorrhagic nephritis conducing to the death of the animal occurred. Enlargement of the glands is not marked. If a very attenuated parasite be injected infection may not take place ; the animal is not protected by such an inoculation.

Rabbits

The parasite has gradually become more virulent for these animals. The incubation period after subcutaneous inoculation is longer than after intraperitoneal or intravenous inoculation. An incubation as short as four days and as long as fifteen days has occurred. The average after intraperitoneal injection is nine days, after intravenous four to seven days. Some animals have died in twenty-six to thirty-five days after intravenous inoculation, showing parasites in large numbers in their blood. The more chronic form of the disease can last from seventy-eight to one hundred and fifty-seven days.

These animals show a pronounced periodicity, the parasites being often absent from their blood for four to nine days at a time, to reappear in small numbers and gradually increase to one to ten to a field. Large numbers of trypanosomes to a field, as in the guinea-pigs, are rare. In acute cases the number of parasites is somewhat increased, and they are almost constantly present. In both the acute and chronic types of the disease anaemia is a very marked symptom. Soon after the appearance of the parasites in the peripheral blood the anaemia is noted, this rapidly advances, so that the blood is of a very pale serous character. At the same time a marked loss of weight occurs. In the chronic cases the animals may become mere skin and bones ;

a loss of four hundred to six hundred grammes is not uncommon in these cases. The coat becomes rough. Oedema of the posterior limbs and base of ears may occur to a certain extent. Discharges from the eye, nose, and genital orifices are rarely seen, and the animal never presents the wretched appearance so often seen in rabbits infected with Nagana, Caderas, etc., diseases. Coincident with the appearance of the parasites the temperature rises, and after continuing so for twenty-four hours falls, after that it may become irregular, being often very elevated, and towards the end becoming subnormal. The spleen is enlarged, but not more so than in rabbits dying from Caderas, etc. The glands may be somewhat enlarged, a few are often haemorrhagic, congestion of the organs is often seen.

Monkeys

A *Cercopithecus callitrichus*, a *Macacus rhesus*, a *Jew monkey*, and a baboon, *Cynocephalus sphinx*, have all been successfully inoculated with the disease. The incubation in the *Calothrix* was four days, the duration lasting over one hundred and sixty days. The *Jew* monkey developed the disease on the sixth day, and died seventy-five days after inoculation. In both these animals the parasites were never present in very large numbers until a few days before death. The *Calothrix* showed more parasites and exhibited more symptoms than did the *Jew* monkey. Anaemia, loss of weight, and irregular temperature were noted in both these cases. A baboon, *Cynocephalus sphinx*, ♀, was inoculated intraperitoneally with a large quantity of virulent blood from a dog which had died from the disease on February 22, 1904. On April 18 a rise of temperature was noted, but no parasites could be found. On May 12 eight parasites were found. Unfortunately a daily examination of the blood could not be made, therefore no definite incubation period can be given. From that time the animal rarely showed parasites in the blood until July 4, when they were nearly always present, though in small numbers, up to its death on September 13. The temperature was irregular. Loss of weight was very marked, especially when the parasites were first seen. Anaemia was not a prominent feature of the disease. A *Rhesus* inoculated with some of the baboon's blood developed the disease on the seventh day. Parasites were hardly ever seen, and it finally died from dysentery. A rabbit inoculated intravenously developed the disease after an incubation of eleven days, it is still living, and appears to have recovered from the disease. Two guinea-pigs inoculated at the same time became infected, but never severely, and died from an epidemic of broncho-pneumonia. The serum of this baboon was slightly more agglutinative to the parasite than ordinary baboon serum. Its serum was not protective.

Post-mortem.—The autopsy of the *Calothrix* showed an enlarged firm spleen, glandular enlargement was general, but not very marked. The baboon showed a slightly enlarged spleen and a little enlargement of the glands. The microscopical examination of the brains and organs is referred to by Dr. Breinl.

Cats

Four cats and two kittens have been inoculated. The disease in the adult cat is of a chronic nature. The incubation, after intraperitoneal inoculation, is from twelve to fourteen days. The duration of the disease in three of the animals was nine to ten months, one slightly smaller cat died at the end of forty-six days. The disease pursues a chronic course, the parasites are prese it only in small numbers, and often are absent for intervals of two to eleven days. The incubation period and the rise of temperature occur together (in one cat, 105·2° F.), after that the temperature falls somewhat but is exceedingly irregular ; after some time the temperature, though remaining irregular, does not rise so high as it did at first. Before death a fall in temperature to subnormal is usual. Loss of weight occurs but the weight is very often regained temporarily ; a month or so before death the loss of flesh is more marked. Anaemia is present but not very marked or progressive. A discharge from the eyes may occur, and in one case oedema around the vulva was noted. The coat is roughened. Two cats which became pregnant after infection aborted. The foeti were examined but no parasites could be found. Small rats inoculated with blood from them never became infected.

A kitten became infected at the end of seven-and-a-half days and died in twenty-three days, the parasites were more constantly present and in greater numbers. Anaemia and loss of weight were marked. Discharge from the eyes and nose was noted. A seeming partial paralysis[1] of the posterior limbs was remarked four days before death. The temperature rose on the appearance of the parasites and remained high but irregular until death.

Post-mortem.—The spleen in the acute form is enlarged. In the chronic form it is only about one-and-a-half times enlarged. The glands are not very big; some few may be haemorrhagic, all contain much juice.

Dogs

Many dogs and puppies have been used. The average incubation after intraperitoneal inoculation is four to eight days, death has taken place in ten to nineteen days. For puppies the incubation is about the same, but the duration appears somewhat prolonged—nine to twenty-six days. The parasites are continuously present in the peripheral blood and increase in numbers, to diminish for a few days. They then begin to augment and usually continue to do so up to or just before death. Loss of weight and anaemia are prominent features. The temperature, after an initial rise, becomes irregular or sometimes continuous ; before death it is usually subnormal.

Dutton and Todd[2] brought back an adult bitch, Experiment No. 11, inoculated October 16, 1902, from Horse I, and infected six days later. This animal, August 31, 1903, was well and strong, its blood non-infective. It is still living, no parasites are

1. While the paralysis is noted no importance is attached to it as young infected animals of all species exhibit such a symptom.

2. Liverpool School of Tropical Medicine, Memoir XI

seen, its blood in amounts of 2·0 to 3·0 c.c. is non-infective. While in England it has been bred and its pups used to see if they were less susceptible than other pups to trypanosome infection. The results show that they acquire the disease quite as easily as other ones, and succumb in the usual time. The serum from the bitch has no protective properties.

Post-mortem.—The spleen is often two to three times larger than normal, the substance is dark and friable, sometimes it may be almost deliquescent. The glands are enlarged, some may be haemorrhagic. Peritoneal exudate is scanty, the pericardium may contain a great deal of fluid. The exudates usually contain a fair number of trypanosomes.

Sheep

A ram was inoculated intraperitoneally. On the sixteenth day the temperature rose slightly and at the same time parasites were seen in the blood. Ten days later ten to forty trypanosomes to a field were noted. These continued in large numbers for the next seven days. The numbers then fell and remained scanty, though continuously present, to death, which took place eighty-four days after inoculation. The anaemia was constant and the animal rapidly became thin. Appetite continued good. No other symptoms were noted. The animal suddenly died during the night. As it was in the height of summer decomposition commenced quickly. The spleen was small, the glands were not markedly enlarged. No oedema or petechial haemorrhages could be found.

Goats

Goat, Experiment 35, inoculated by Dutton and Todd from Horse I, November 28, 1902, remained infected up to March 20, 1903, it was then sent to England. In August, 1903, its blood was negative to microscopical examination, and non-infective even when injected in amounts of 2·0 to 3·0 c.c. It was reinoculated in October and became infected. The temperature rose and remained irregular. Death occurred after inoculation. The parasites were always very scanty until just before death. The autopsy disclosed enlarged spleen and glands, some few of the latter being haemorrhagic. Anaemia was noted a few days before death.

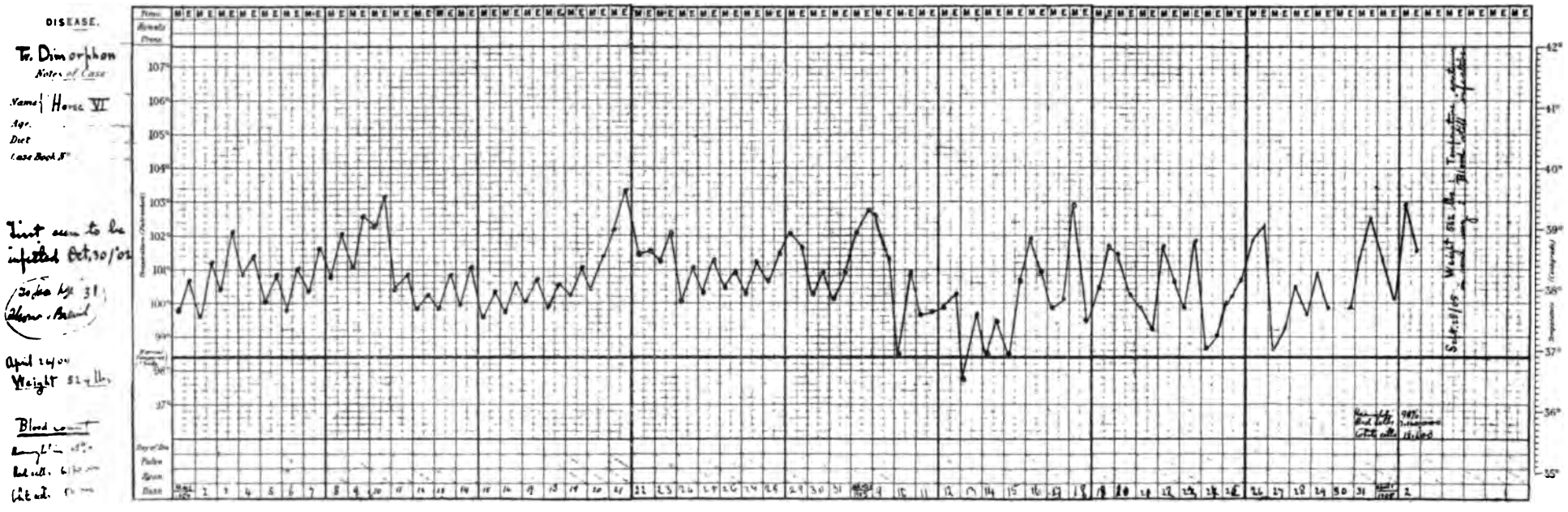
Goat 10, inoculated by Dutton and Todd, October 15, 1902. Appeared to have apparently recovered in October, 1903; in March, 1904, it was killed by accident while being bled. A guinea-pig, inoculated with 100 c.c. of pure heart blood, became infected after a prolonged incubation. The serum from these goats did not cause any permanent agglutination.

Horse

The stallion,[1] naturally infected in the Gambia and sent back by Dutton and Todd, has been under almost continuous observation from October 30, 1902. Since August, 1903, it has been bled at regular intervals, and its blood inoculated into

1. *First Report of the Expedition to Senegambia*, p. 31.

CHART V



to face p. 30

susceptible animals. After two years and five months it is still alive and apparently in good health. *Its blood is still infective to rats and mice*, but the amount necessary in order to secure an infection has had to be increased. Formerly a few drops of blood were required. At present 1·0 to 1·5 c.c. of pure blood is needed to infect a medium-sized rat ; rabbits require 3·5 c.c. of blood. The blood still preserves the characteristic agglutination of the corpuscles. Very occasionally a trypanosome has been found after long continued examination. The temperature has lost the markedly irregular type which characterises the disease. The animal has put on weight and has become accustomed to the change of climate. No symptoms of oedema, etc., have been noted. Blood counts :—April 8, 1905, reds, 7,160,000 ; whites, 17,600 ; haemoglobin, ninety per cent. Its blood no longer causes any more agglutination than does normal horse serum ; the protective properties are nil. The urine is normal.

From the above records it will be seen that observations on the disease are in accordance with the findings of DUTTON and TODD and LAVERAN and MESNIL. The disease in horses can become chronic and the animal may live for some time, probably a cure will result in such a case as the above. From Goat 10 and Horse 6 cases it is evident that animals in which the disease is markedly chronic for some time, will hardly show any evidences of infection. The blood, in amounts of even 1·0 to 1·5 c.c., is non-infective, and when inoculated in larger amounts, 3·0 to 10 c.c., the incubation period is prolonged.

It is not our intention to enter into a detailed description of the parasite as the morphological characteristics have been well pictured and described by DUTTON and TODD, LAVERAN and MESNIL. The parasites have been examined in both fresh and stained specimens from the blood of all the species of animals used in the investigation. In no instance has the long form, described by DUTTON and TODD and possessing a long thin body and a long flagellum, been seen ; in this we are in accord with LAVERAN and MESNIL. The long form is known to have been seen in the blood of animals, but it disappeared early in September, 1903, when the research work in Liverpool was begun. From a study of the films the forms described by DUTTON and TODD (Pl. I, Fig. VII, VIII), and LAVERAN and MESNIL (Fig. XX), are those seen by us. Granules have been seen in many specimens, but not so numerous as in other species of trypanosomes. The agglutination of *T. dimorphon* is a very noticeable feature. As pointed out by LAVERAN and MESNIL it is especially remarkable in the blood of rats and mice shortly before death, it is also well seen in rabbits and guinea-pigs under the same conditions. In making agglutination experiments with serum, etc., it is therefore necessary to avoid such blood.

IV. *TRYPANOSOMA EQUIPERDUM.* DOURINE--MALADIE DE COIT

This disease has been under observation for some time. Two rabbits which had been inoculated from a dog were received. One rabbit was killed, and the whole of its blood used to inoculate a pup and a rabbit. The pup showed parasites in its blood on the eleventh day, and death occurred on the forty-ninth day, many parasites were found in its blood and in the peritoneal and pericardial exudates. Since that time the strain has been kept running through young puppies and rabbits.

Eight puppies have been used. The incubation period has been from four-and-a-half to eleven days, the average being six-and-three-quarters to seven days. The duration of the disease is from twenty-two to sixty-four days, the average period being from five to six weeks. At first it is extremely hard to find trypanosomes in the blood, but after the third week they quickly increase in numbers, and continue to augment so that eight to ten to twenty-five or more to a field are present. Divisional forms at first are very few in number, but soon abound in the peripheral blood. Profound anaemia occurs coincidently with the increase of parasites. Loss of weight is a marked feature. A characteristic feature of this disease is the oedema of the posterior limbs ; an oedema around the genital organs, extending along the abdomen, is also often very marked, and lasts for a few days or remains till death. In some cases the only oedematous areas are about the site of inoculation. Petechial plaques with loss of hair over these areas have been noted in two cases. Purulent discharge from the eyes and nose very often occurs. There is usually a slight discharge from the genital organs. Nervous symptoms have been rarely seen. In only one pup has a partial but true paralysis of the posterior extremities been noted. This pup developed a halting gait, which steadily became worse, due to an increasing paralysis of the right hind leg. The reflexes were lessened, and towards the end almost completely absent. The autopsy on this pup revealed nothing special, its cord and brain have not been microscopically examined.

One adult dog developed the disease in thirteen days. For a period of three weeks the oedematous condition around the genitals was very marked, the sheath being particularly affected. In addition, small unconnected oedematous areas were present on the inside of the thighs. These oedematous patches contained fluid, in which were very many trypanosomes. The dog lost flesh and became very anaemic, but recovered after two-and-a-half months. Four months later it was killed, and a pup inoculated from its blood developed the disease.

Rabbits

Rabbits inoculated subcutaneously develop the disease in six to eleven days and die in twenty-four to one hundred and eight days, some have lived over six months. The parasites are very scanty during the whole course of the disease. Oedema of the ears and genital organs, very often persisting and increasing, is marked. Loss of hair has been noted on one or two occasions, usually at the base of the ears and around the eyes and nose. The genital organs are swollen, the urethra or vaginal mucous membrane is pinkish and covered with catarrhal exudate. The discharge is often pronounced. The discharges from the eyes and nose are not so marked as in rabbits infected with the parasites of Nagana or Caderas. Examination of these discharges always proved negative.

Rats

Twenty-three rats have been inoculated with large numbers of the parasite ; in only two have parasites been found after the fourth day. One died on the eleventh day, the other on the eighteenth. In neither case could the autopsy be done early, and therefore the negative finding is not to be considered. The spleen and glands were small. A rabbit inoculated with the blood and extract of mashed organs of one of these rats never developed the disease.

Cats

One adult female cat was inoculated at the same time as a pup ; on the ninth day the temperature rose but no parasites could be found. The experiment proved negative.

Guinea-Pigs

Four have been inoculated with negative results.

Goat

A goat inoculated subcutaneously with ten c.c. of heart blood and twenty c.c. of peritoneal exudate never developed the disease. Its temperature remained normal, and its blood negative ; a pup inoculated from it never became infected.

Post-Mortem Appearances

The autopsies on the pups showed a moderately enlarged spleen and enlarged glands, some few of which were haemorrhagic, these being found usually in the retro-peritoneal, inguinal, and axillary groups. The blood was very serous and pale, and usually contained numerous parasites. The bone marrow was pale and often very soft. The pericardial sac contained some slightly clouded fluid. The peritoneal cavity usually contained a large quantity of clear watery or straw-coloured fluid.

These fluids contained trypanosomes often in large numbers. The oedematous patches of tissue contained trypanosomes, often present in greater numbers than in the blood. Petechial patches, the size of a pin's head, were found on the pleural surface of the lungs. The brain and cord did not show any marked macroscopic changes, very often there was considerable oedema around the lumbar region of the cord. The cornea was sometimes opaque.

The autopsies on the dog and rabbits and cat showed no special features. An account of the histo-pathology of the nerve tissues and organs will be found in Part II. Divisional forms of the parasites have been found in all exudate fluid, exudates and juices from the organs. If the autopsy is done immediately after death large numbers of remarkably fine divisional forms will be found in the exudates. These forms rapidly lose their contour if the animal has been dead some hours.

TRYPANOSOMA EQUINUM. MAL DE CADERAS[1]

A large number of animals have been inoculated with this parasite.

Rats—White, Black, and Grey

Rats are easily infected. Subcutaneous inoculation causes the parasites to appear in two-and-three-quarters to three-and-a-quarter days. Intraperitoneal, two to two-and-a-half days. The average duration is six to eight days. In some few cases parasites have been found in the tail-blood in thirty-six hours, the blood is infective at this time.

Mice—White and Grey

Mice are infected somewhat earlier than rats. The duration of the disease is slightly shorter.

In both rats and mice the incubation period and the duration of the disease is lengthened if the inoculated fluid is poor in trypanosomes. Sometimes one notes a temporary lessening in the number of the parasites, this usually lasts for one to three days, sometimes longer. The parasites then increase and usually continue to do so until death. Rats and mice inoculated with blood from animals undergoing treatment with arsenic, especially when the treatment has only been in force for a short period, develop the disease after a prolonged incubation. The parasites may appear for a few days and then disappear to remain absent or once more reappear, such animals are not protected. Loss of weight is noted both in rats and mice when showing marked infection. In some of the rats inoculated with the attenuated parasite the loss has been less marked and of only temporary duration.

Rabbits

The average incubation period has been four to five, sometimes six days. The duration has been sixteen to fifty-nine days. In a few of these animals the parasites have been fairly numerous in the peripheral blood, as many as ten to fifteen to a field being recorded. The majority of the infected rabbits duly show the parasites in very scanty numbers. The parasites are usually found in the blood when the temperature rises. Cases are recorded with a preliminary rise on the fourth to fifth day to 106° to 107° F. The fever is irregular, usually not passing 104° F. A few days before death the rise of temperature may be very marked, death occurring usually with a fall in the temperature. Loss of flesh and a somewhat marked anaemia is noted; this latter symptom is not so marked as in rabbits inoculated with *T. dimorphon* or *T. gambiense.* Oedema at the base of the ears and around the genitals is often marked. Loss of hair around the eyes, nose, and base of ears is usually pronounced.

1. We have been able to study this parasite through the courtesy of Professor Laveran.

The testicles are generally swollen, there usually is a discharge from the penis or vulva. The discharge from the eyes and nose is very severe, causing the animal to present a very miserable state. It has been noted that in treated animals the discharge and oedema quickly disappear, and the hair begins to grow over the bald patches.

Guinea-Pigs

Subcutaneous inoculation causes the parasites to appear in eight to ten days. Intraperitoneal injection takes six to eight-and-a-half days. The duration of the disease may be from twenty-two to one hundred and fifty-three days, the average period being seventy-five to ninety days for adults, and thirty to forty-six days for young guinea-pigs (three hundred grammes). The majority of the animals have shown parasites in their blood almost constantly ; occasionally the organisms nearly completely disappear. This is especially so after a marked increase in numbers. Small guinea-pigs usually harbour large numbers of trypanosomes in their blood. The appearance of the parasites in the peripheral blood is coincident with a slight rise of temperature. The fever then becomes irregular, but not more so than is met with in guinea-pigs infected with Surra, Nagana, etc. Loss of flesh is noted, but is more gradual than in rabbits. Paralysis is rarely observed. Three guinea-pigs showed partial paralysis of the hind extremities, six, three, and one day before death. No guinea-pigs out of forty-three infected and not treated have survived the disease.

Cats

Kittens are more susceptible than adults. The incubation is six-and-a-half to eight days, and death occurs in about thirty to fifty-six days. Adults develop the disease in seven to nine to eleven days, and may die in two-and-a-half to six months. The appearance of the parasites in the general circulation is associated with a rise of temperature. The chart of an infected animal showed an irregular rise and fall with sharp rises to 104° to 105° F. With kittens the temperature usually remains high. The adults rarely show the parasites in their blood, but the agglutination of the corpuscles is often very pronounced. Kittens usually have the parasites almost continuously in the peripheral circulation. The parasites usually are found in the blood at the time of the sharp rises of temperature. Oedema is rarely observed. Discharges from the eyes, nose, and genitals are rare. The autopsy shows a certain enlargement of the spleen. The glands are somewhat swollen. Haemorrhagic glands are rarely seen. In the kitten the gland juice frequently contains many parasites.

TRYPANOSOMA EVANSI. SURRA

This strain was also given by Professor LAVERAN, its origin is from one of the infected animals in the epidemic which ravaged the island of Mauritius.

A guinea-pig showing a severe infection was received, from it a series of animals have been inoculated.

RATS—WHITE, BLACK, AND GREY

These animals are as susceptible to Surra as to Nagana. The incubation after subcutaneous inoculation has been three to four-and-a-half days, after intraperitoneal injection two-and-three-quarters to three-and-a-half days. The average duration is about five to seven days after the appearance of the parasites in the peripheral blood.

MICE—WHITE AND GREY

The incubation after subcutaneous inoculation is about three-and-three-quarters to four days, after intraperitoneal inoculation about three days, duration six to eight days after the infection declares itself. In both rats and mice the parasites usually continue to regularly augment, so that as death approaches large numbers of parasites are found in the peripheral blood. Careful observation has shown that occasionally a decrease in the number of the parasites present in the tail blood is to be noted, it is, however, only transitory, as the numbers quickly increase, so that at death the blood will swarm with the organisms. In rats and mice inoculated with attenuated trypanosomes or with the blood of less susceptible animals, the parasites being fewer in number, the incubation period is lengthened, and the course of the disease prolonged. As in Caderas and the other trypanosomic diseases rats and mice inoculated with blood from animals undergoing treatment, especially where the parasites are still present, though in very scanty numbers and degenerated, may only show a few parasites in the blood. In such cases the parasites are so attenuated that they do not increase rapidly, and the animal acquires a chronic form of the disease. These animals frequently recover, but they are not immune.

RABBITS

The incubation period varies. After intravenous inoculation the parasites appear in three-and-a-half to five-and-a-half days. The presence of the parasites in the peripheral blood is usually accompanied by a slight rise of temperature. Sometimes the rise is marked, being 106° to 107° F., such a high temperature is usually associated with a severe infection. The fever may be marked and of an irregular type, or there may

be hardly any noticeable rise during the course of the disease. The temperature may sharply rise, this is usually in conjunction with an increase in the number of the parasites. The parasites may be noticeably scanty, or they may, as we observed in a few cases, be present in large numbers—ten to forty to a field. Death is usually preceded by a fall in temperature, sometimes the parasites are almost absent before death, at other times they are largely increased in numbers.

The symptoms so often associated with Nagana and Caderas are seen in rabbits infected with the Surra parasites. The oedema of the ears, perineum, the swollen testicles, the tumified vulva, the discharge from the penis, eyes, and nose ; all these are present to a greater or less extent. Anaemia is present but not markedly so. Loss of weight is a constant feature of the disease. Young rabbits are easier to infect than adult ones. The duration is lessened, the parasites are generally more numerous.

Guinea-Pigs

Incubation is six to eight days. Duration, forty days to two to four months. The parasites at first are present in scanty numbers, later in the disease they increase. Sometimes they may almost disappear from the blood and remain so for some time to augment once more. The rise of temperature is usually associated with the appearance of the parasites, the temperature is never very high. Anaemia is not a prominent feature. Loss of weight occurs, being especially marked when large numbers of trypanosomes are present in the blood for some time. Oedema has been noted on a few occasions. No animal has recovered without treatment.

Cats

After intraperitoneal inoculation infection takes place in eight to twelve days. The infection is usually long continued, the parasites are scanty in the blood. The chronic form of the disease in this animal may last six-and-a-half to eight months. There is a certain amount of anaemia associated with a slight loss in flesh ; towards the end these symptoms become more pronounced. A discharge from the eyes and nose has been observed. One cat showed quite a pronounced oedema of the perineum. The disease in kittens manifests a severer form, the parasites are usually numerous, and the duration of the disease may be only four to six weeks. The autopsy shows in these chronic cases very little of interest. The organs appear very little changed. The peritoneal and pericardial exudates, if any, are scanty and usually no trypanosomes can be found. Kittens show an enlargement of the spleen and glands, their blood and peritoneal and other exudates usually contain parasites.

Dogs

Several animals have been inoculated. In all cases after subcutaneous injection the parasites have been found in the peripheral blood in seven to nine days. At first

the parasites are scanty. After a few days marked exacerbations, persisting for a few days, occur ; or the parasite may be scanty until only four to six days before death. The end can occur in sixteen to thirty days, depending on the virulency of the parasite and the method of inoculation. The animal at first presents very few symptoms, but when once the parasites are increasing rapidly a very decided and rapid anaemia, together with emaciation, occurs. The animal continues to lose strength, death is usually preceded by a subnormal temperature. The usual *post-mortem* lesions met with in dogs dying from acute trypanosomic disease are met with.

V. BACTERIOLOGICAL EXAMINATIONS, MORPHOLOGY, GLAND PUNCTURES, AND BLISTERS

Bacteriological Examinations

Cultures of the blood and cerebro-spinal fluid were made from two of the fatal cases of sleeping sickness and several times from ' Native Fever ' cases.

The blood was obtained from one of the arm veins by means of a Roux Syringe —the cerebro-spinal fluid through lumbar puncture. The media used consisted of broth, agar and gelatine of various alkaline or acid reactions. Sugars or peptone, salt, etc., were contained in some media, in greater or less proportions, and were absent from others. Large Ehrlenmeyer flasks containing fifty to two hundred c.c. of nutrient media were inoculated with some of the blood or cerebro-spinal fluid. Anaerobic and aerobic methods of cultivation were employed. The inoculated media were incubated at 18°, 23°, and 35° C.

From Kitambo. Cerebro-spinal fluid, one flask and two tubes showed small growths of *Staphylococcus epidermidis albus.* These were the first cultures inoculated.

From Tomi. All cultures remained sterile. All cultures were kept under observation for two months.

From some of the animals infected with the cerebro-spinal fluid of the two sleeping sickness cases cultures were made at death, the results were negative.

At the *post-mortem* on the two sleeping sickness cases, cultures were made. The blood and cerebro-spinal fluid cultures remained negative. The spleen and liver showed in Kitambo *B. coli communis* and putrefactive bacteria.

From Tomi. No growth.

Variations in the Morphology of the Parasites

From a comparison of stained preparations of *T. gambiense* made during the course of the disease no differences can be made out. The parasites in the sub-inoculated animals seemed somewhat larger. This seemed to be the case with many trypanosomes.

The parasites in the three cases of Trypanosome Fever compared with those found in the blood of the two sleeping sickness cases show no determinable differences. The organisms found in the cerebro-spinal fluid of one case showed a more vacuolic condition, the parasites appeared slightly more rounded at the posterior end. None of the amoeboid forms described by Castellani were seen. The trypanosomes in the blood of the same case did not show the vacuoles so markedly. The observance of many slides of blood, which is often very serous, and of exudates,

causes one not to rely on the presence of vacuoles. They often appear to be present in trypanosomes living under unfavourable conditions. In animals treated with arsenic, vacuolic forms can be seen. If non-vacuolated trypanosomes be injected into the peritoneal cavity of a guinea-pig and some of the fluid withdrawn after a few hours, many trypanosomes containing large vacuoles can often be seen. This is especially so with *T. gambiense*. The vacuoles seem more accentuated in stained films than in the fresh specimens. As LAVERAN points out this is especially observed in slides made from the cerebro-spinal fluid or from very serous blood. Films from such fluids take longer to dry even if the slide be warmed to blood temperature beforehand.

If in a heavily-infected animal the parasites seen in its peripheral blood be compared with those met with in the organs, bone marrow, and large vessels, no differences can be made out. A series of animals were killed and the numbers of parasites present in the organ juices, heart, and large blood vessels compared with the peripheral parasites. In all cases the peripheral blood contained more, the average number of parasites in the different organs were the same. The various groups of glands did not contain more. On comparing the numbers found in the organs of animals which had died from the disease, at from one to twelve hours after death, the results have varied. If the blood be very serous more parasites will be found there than in the organ juices. Sometimes the bone marrow or one organ will contain more than the others. The brain and cord contain fewer than do the organs.

GLAND PUNCTURE—PARASITES IN THE GLANDS

A study of the number of the parasites in the glands as compared with those met with in the blood have not confirmed GREIG and GRAY'S observations. From one native superficial glands were removed. Twice were glands taken at a period when the blood was negative to ordinary examination, and once when the blood contained a fair number of parasites. After excision the glands were immediately dried, the surface cauterized, and the gland juice withdrawn. When the blood was negative the glands did not show any parasites; on the occasion when parasites were observable in the peripheral blood the gland juice contained a less number of trypanosomes. Gland juice was also removed by puncture with the same results. Similar findings are to be recorded with glands excised from or punctured in animals infected with the disease. Unfortunately, monkeys, rabbits, guinea-pigs, and rats do not usually have very enlarged glands, but in the other animals no better results can be shown. Gland puncture of two donkeys infected with *T. gambiense* and with very few parasites in their blood have not proved of use in the routine examination of the animals, though the glands were enlarged and easily palpable. DUTTON and TODD have made use of gland puncture with very satisfactory results.

Glands were removed from lightly infected animals—strains, *T. gambiense*, *T. dimorphon*, *T. evansi*[1]—showing parasites in only scanty numbers in the blood. The glands were immediately mashed up with citrate solution and the extract injected into the peripheral cavity of susceptible animals. Eleven experiments were done. In nine the animals were infected only after a prolonged incubation ; in two cases the animals never showed parasites. Controls inoculated with blood and citrate became infected in a shorter time.

Occurrence of Parasites in Blisters

One of the experimental animals having a blister the exudate from the small bleb was noticed to contain a few trypanosomes. By means of cantharides plasters small blisters were made, and the serum was examined from two of the natives and some of the animals. The results were not encouraging. On occasions when the blood was negative the fluid from the blisters was also negative, and on days when the blood was positive only a very small number were seen in the exudate. Sufficient exudate was collected and mice inoculated. After an incubation period of twenty-one days in two out of four mice inoculated intraperitoneally with 0·5 and 1·0 c.c. parasites were seen in small numbers.

Diagnostic Methods

Much attention has been directed to find some method of diagnosis in cases where the blood and gland-juice examinations fail to show parasites; in cases of failure, and when the centrifuged blood is also negative, resource must be had to inoculation. Dutton and Todd record a certain number of their inoculated rats failing to show signs of infection, even when inoculated with blood verified to contain motile parasites. All the rats inoculated from the natives have shown parasites in their blood, yet in some cases the incubation period has been prolonged to thirty-two days. Rats are in many ways unsatisfactory, as the parasites may be present in only very small numbers. Inoculation of larger animals, rabbits, guinea-pigs, and cats are also open to the same objection. Monkeys are most suitable, since they are easily infected ; then come young pups, kittens, rabbits, and adult dogs. Advantage was taken of the periods when the trypanosomes were absent from the peripheral blood of the natives. If after several days the blood still remained negative, small quantities of blood, ten to thirty c.c., were withdrawn from one of the superficial veins and, combined with citrate solution, the mixture was

1. While experimenting with *T. brucei* in Professor Kitt's laboratory, Veterinary School, Munich, and at McGill University, a series of animals were inoculated with extracts from the mashed up organs, glands, bone marrow, etc., of infected animals. Infection quickest with blood mixture, then bone marrow, spleen and liver, kidneys and adrenals, glands, brain, cord. A repetition of the above with *T. gambiense* and other trypanosomes has given almost the same results. Pieces of organs removed aseptically and inserted whole under the skin or in the peritoneal cavity of rats and guinea-pigs have infected the animals after a more or less prolonged incubation period. Pieces of organs left under the skin or in the peritoneal cavity for some hours and then taken out and examined showed trypanosomes more degenerated and in less numbers than in control pieces examined. Celloidin sacs filled with blood containing parasites and placed in the peritoneal cavities of rats and pigs for a time show the usual degenerating, deformed trypanosomes.

inoculated into the animals. These animals became infected, though the incubation period was lengthened. After the appearance of the parasites the disease ran its natural course, and the animal succumbed to the infection in almost the same time as others which had been inoculated with blood in which the organisms were seen.

Monkeys of 1·5 to 2·5 kilo. weight inoculated intraperitoneally with 5·0 to 8·0 c.c. of apparently negative blood always became infected. Kittens can receive two c.c., pups, two to four c.c. of blood intraperitoneally without untoward symptoms developing, and show parasites usually in nine to fourteen days. The kitten and pup are especially useful for studying the disease as they quickly show the parasites in large numbers, and periodicity, particularly in kittens, is not a marked feature. Dogs are also useful, but the number of parasites observed may for a considerable period be small, and the possibility of the unskilled observer passing them by is enhanced.

The Cultivation of Trypanosomes

Novy and McNeal in a series of papers emanating from Professor Novy's laboratory, have shewn that it is possible to cultivate some of the trypanosomes infecting animals, *T. lewisi*, *T. brucei*, *T. evansi*, and a numerous series in birds. We have been able to cultivate *T. lewisi* and *T. brucei* on the media proposed by these investigators. Our attention has, however, been directed more to the artificial cultivation of *T. gambiense*, *T. dimorphon*, and the other pathogenic trypanosomes. If the results must conform to Koch's postulates, then we must admit we have failed. Various strains of *T. gambiense* are capable of being cultivated in a modified blood agar medium and kept alive for sixty-eight days by transference from tube to tube or through flasks ; after the seventeenth day of cultivation we found it no longer capable of infecting a susceptible animal, no matter what amount of culture was used. The trypanosome loses its form to a great extent, its staining reaction is altered and it finally dies. Numerous trials have been made with various changes in the composition of the media but without success. Guided however by the history of the numerous failures of Novy and McNeal before their efforts were rewarded, we believe that our successors will be able to cultivate these parasites with greater success than we have had. That the task is a difficult one is evidenced by the fact that of the many trypanosome investigators only one in addition to those working in Novy's laboratory has been been able to publish a successful result, and this only a repetition of a former investigator's work.

A synopsis of our attempts is included here in the hopes that they will act as a spur to the investigation of this most important subject. As the result of our experience, we feel that the undivided attention of the research worker is necessary to secure success.

After cultivating *T. lewisi*, attention was paid to *T. brucei*, using blood agar in the proportion 2 : 2 and 2 : 1 ; the third attempt proved successful. This culture was run through five generations before it died out from the effects of contamination. Efforts were then directed to *T. equiperdum*, *T. equinum*, and *T. evansi*. The culture of *T. equiperdum* was difficult, as the parasites were not present in large numbers, but at the fifth attempt two tubes out of nineteen inoculated with blood and exudate fluid from a pup, showed motile trypanosomes on the eleventh day. One tube was infected with heart blood, the other with oedema fluid obtained from the subcutaneous tissue of the thigh. The heart blood tube showed evidences of growth and the formation of reduplication forms, but only one in thirty fields, while at the time of inoculation large numbers of these forms were seen. The oedema fluid on the seventeenth day showed many divisional forms undergoing division. These forms were agglomerated together into clumps, not rosettes, as after the manner of *T. lewisi* in culture ; many single well-preserved forms were seen, but the motility of all was much lessened. Many deformed and motionless, granular, degenerated trypanosomes were seen. At this time a pup used for keeping the strain running in the laboratory died. Resource had therefore to be made to the cultures. The total contents were taken and injected into the peritoneal cavity of a puppy. On the eleventh day after the inoculation a slight rise of temperature was noticed, but no parasites could be found. Nineteen days later, *i.e.*, thirty days from date of inoculation, no parasites having been seen it was killed and the whole of its blood inoculated into another pup. This pup after a prolonged incubation became infected. The medium used in this instance was chicken-broth, in the proportion of meat one to water two with 0·5 per cent. peptone and 0·25 per cent. sea-salt added, and rendered faintly alkaline, agar 2·5 per cent., and defibrinated rabbit blood 2 : 1.

T. equinum.—Many failures with this parasite have to be recorded. Cultures were made on various strengths of beef-broth agar, with different proportions of blood, peptone, etc., with no result. Rats, mice, rabbits, and guinea-pigs were used, the animals being killed at all stages of the disease. The trypanosomes quickly died off. One effort yielded a positive result on a chicken-broth agar tube of similar composition to that used for the *T. equiperdum* culture. It was one tube out of twelve inoculated from a rabbit, which had died about three-and-a-half hours before the inoculation of the cultures. The trypanosomes were in the average about one to eighteen fields. The tubes examined showed no growth, after the twelfth day only dead parasites were found. These tubes were left in the incubator at 22° C., and were not examined till the twenty-ninth day, when the majority were found to be overgrown with mould. One was not and it contained a few trypanosomes, many of them being paired, looking apparently healthy with fair motility. A few transplants were made into fresh blood agar tubes, and the rest of the culture was used to inoculate a rat, which became infected after an incubation of eight-and-a-quarter days. The transplants never grew. Further efforts failed to produce cultures.

T. evansi.—Here, as with the other trypanosomes, numerous cultures from the blood of different animals have been made from time to time. Seven tubes at 22° C. showed growth up to the nineteenth and twenty-second days, but these were contaminated with bacteria. One tube showed a growth on the twenty-ninth, another on the thirty-third, still another on the thirty-seventh day. These three latter were all room temperature cultures, the efforts to transplant the twenty-ninth and thirty-seventh day cultures proved failures, nor did the original tubes show any more growth when placed in the 22° C. oven, all the trypanosomes quickly dying off. The thirty-third day tube was used for inoculating a rat and a guinea-pig without success; the few remaining drops were used for sub-cultures, but no growth resulted from them. The trypanosomes in these three tubes, especially that of the thirty-third day, resemble the description which NOVY, MCNEAL, and HARE have given of their efforts to cultivate the Surra trypanosome of the Philippines. The parasites were few in number, were all single and actively motile, the majority showed dark granules at their posterior ends, and resembled the first two drawings of Fig. 1 and the lower of Fig. 2 (NOVY, etc.) The flagella seemed longer and thinner than in the normal parasites obtained from an infected animal.

T. gambiense.—The difficulties attending the cultivation of this organism are many. The parasite does not as a rule appear in great numbers in the blood. If an animal is infected with the parasites of Nagana, Surra, etc., once they appear they will augment from day to day until the blood fairly swarms. With *T. gambiense*, on the other hand, rats rarely show very large numbers in their blood, and periodicity being a feature of this disease, the parasites present one day will be found to be absent the next day. This feature is constant in all the animals inoculated. Monkeys, pups, and kittens, however, usually have more parasites in their blood than do the other animals. Advantage as far as possible has been taken of this fact. With the ordinary blood-agar medium no growth takes place, and the trypanosomes die off at once. Veal and chicken-meat infusion has been found to be more suitable than beef infusion. One to 1·5 per cent. peptone with 0·25 to 0·5 per cent. sea-salt is added and agar from 2·5 to 3·5 per cent. Various animals have been used for bleeding—horse, goat, sheep, dog, and rabbit. Almost as satisfactory results have been obtained from the blood of the goat and sheep as from that of the rabbit. The blood has been used in various proportions—2 : 2, 2 : 1, 3 : 1—the most satisfactory proving 2 : 1 or 3 : 2.

The trypanosomes for the first few days appear to tolerate the media, but after the fourth to seventh days numerous degenerated and dead ones are seen. In cultures kept at 22° to 23° the tubes will at the fourteenth to sixteenth day either contain dead trypanosomes or a few partially motile ones. These motile ones may present the normal outline of the parasite, but granules are usually present in the anterior portion of the body. The macronucleus of these forms is often of a granular appearance, staining unevenly, and sometimes fissure-like breaks may

occur. The flagellum is usually shorter, and it and the undulating membrane when stained appear thinner. The motility of some of the forms is accentuated, but usually the motion is markedly lessened. Many parasites are globular and show whitish areas, apparently vacuoles, in the body, and these sometimes encroach on the macronucleus. The motility of these forms is nearly always lessened. The trypanosomes usually occur singly or in two, three or four to a group, sometimes clumps of twenty to thirty will be found, the flagella facing outwards ; these clumps are not composed solely of parasites, but consist of a mixture of blood cells, fibrin, and trypanosomes. Rarely after the twenty-fifth day have normal looking organisms been seen, though at this time there may be found dead trypanosomes with perfectly formed outlines and flagella. If from the sixteenth day onwards attempts be made to transplant the motile trypanosomes into fresh media the majority of the tubes will be failures. A few, however, may survive, and in these, after a few days, the trypanosomes will appear to lengthen out and become still more normal looking. The motility is normal, but unfortunately no duplication forms will be seen. No evidences of actual growth occur, and usually by the twenty-fifth to fortieth day from the first generation the organisms will be found dead. Such cultures naturally do not infect animals even when the whole culture is used to infect one animal. On three occasions tubes which on the twelfth to seventeenth days had looked hopeless, with the degenerating parasites increasing each day, have on the addition of fresh culture fluid from another tube appeared rejuvenated ; some of the parasites appearing to lose their dark granules and to become less stumpy. Twice the contents of such tubes have been poured into other tubes, and the parasites have continued motile up to the forty-third day. The parasites of a first generation tube which behaved in this manner were transplanted into another tube, and were alive, though badly degenerated, up to the fifty-sixth day. They were once more transplanted, but twelve days later were all dead. The original tube had contained motile trypanosomes up to the forty-seventh day.

Various strains of *T. gambiense* have been used. Some of the cultures were inoculated with blood in which the short flagellated and vacuolated form, though associated with the longer form of the parasite, predominated. It has been noticed later on in such cultures that sometimes more vacuolated short forms remained or *vice-versa*. This, though it may not be definite, goes to refute the idea which some investigators have put forward that the short vacuolated form is typical of the parasite found in sleeping sickness cases. With the very virulent strain 'E' some attempts have been made to cultivate the parasites. No living trypanosomes have been found after the nineteenth day. These cultures were non-infective for animals.

T. dimorphon.—A great deal of work has been done with this parasite. Tubes of culture media of the same proportions as in the experiments with *T. gambiense* have been used and the animals from which the inoculating material has been taken

have included rats, mice, guinea-pigs, and rabbits. This parasite has naturally a stumpy form and possesses the peculiarity of a marked tendency to agglutinate in animals near death ; this is especially the case in moribund rats and mice. After inoculation of the culture tubes very little is noted for the first three to four days, and the parasites are usually gathered together in large clumps or several of them are agglutinated side by side or by their anterior ends. The motility is not altered, the form is normal and the parasite stains as usual. Cultivated in the room or at 22° C. changes occur after the fifth day, especially in the tubes incubated at 22° C. The parasites become slower and many show granules. Numerous globular and distorted forms are seen. About the seventh to eleventh days the majority are either motionless or dead. However some very acutely rounded or oval forms, pear-shaped and twice as broad and about half as long as the normal ones, are seen. These latter often recall the 'tadpole' shape, and they may become more rounded. Coincident with these changes larger forms appear, which to a certain extent resemble in size and shape cercomonads. The motility of both these forms is remarkable when compared to the ordinary motion of the parasite ; it is very striking to observe them as they whirl into a field and circle round, often lashing out and going to the side of or leaving the field. Their motion is however, usually circumscribed to the one field. Such forms when stained show a badly staining granular protoplasm, a very small macronucleus and micronucleus. Usually the body of the rounded forms contains small chromatin bodies. The flagellum is thin and only faintly stained, the undulating membrane is hard to make out. In order to stain these forms, longer staining is necessary than with normal trypanosomes. If now, cultures containing these bodies be transplanted, very few of them will survive. Cultures containing these forms are non-infective to animals.

Cultures at 22° C. containing normal-looking trypanosomes have proved infective to animals as late as the twenty-third day, but only when injected in large amounts. By sub-inoculations of the cultures of the very active but degenerated forms, it may be possible to keep the parasites alive to the thirty-sixth day. A second generation tube which had been inoculated with a small piece of the surface of a blood agar flask which had been covered with clotted blood, showed two trypanosomes almost dead, but faintly moving on the seventy-sixth day. This tube was sub-inoculated from a 22° C. flask on the nineteenth day and left sealed up from the thirty-second day. No other parasites than these two could be found.

Summary

1. The parasite *T. equiperdum* in blood agar tubes is capable of infecting a pup up to the seventeenth day, when the tubes are kept at 22° C.

2. The parasite of *T. equinum* is capable of infecting a rat twenty days after the inoculation of the tube. There appears to be an actual growth taking place.

3. *T. evansi* in blood agar cultures at 22° C. is living, but non-infective up to the nineteenth to twenty-second day. Three tubes at room temperature contain motile but non-infective parasites on the twenty-first, thirty-third, thirty-seventh days.

4. *T. gambiense* of any strain is capable of living in a non-infective form in a first culture flask up to the thirty-fifth to fortieth days. Cultures in tubes and flasks, which on the twelfth to seventeenth days contained degenerating parasites, have by the addition of fresh culture fluid regained their vitality. Twice the contents of such tubes by transplanting to a fresh tube have lived to the forty-third day. One lived to the fifty-sixth day, and by a second transplant survived up to the sixty-eighth day.

5. *T. dimorphon* in culture tubes and flasks is infective to animals up to the twenty-third day, when infected in large amounts. Sub-inoculated but non-infective cultures remain alive as long as the fifty-sixth day. In one case, two trypanosomes showing motion were found on the seventy-sixth day.

6. In no case has any evidences of a toxine been noted in the inoculated animals.

VII. THE TREATMENT OF TRYPANOSOMIASIS

Experiments have been conducted under this head on all the various trypanosomic diseases described in the previous investigations, and include *T. brucei*, *T. evansi*, *T. equinum*, *T. equiperdum*, *T. dimorphon*, *T. gambiense* (various strains). In 1902-1903, while Governors Fellow of Pathology at McGill University, one of us (H. W. T.) made experiments with sodium arseniate, etc., on animals infected with *T. brucei* and *T. lewisi*. These tentative experiments have now been carried out on a large scale. As in the case of other workers we have tried a very large number of drugs to find them of no value in the treatment of infected animals. WENDELSTADT, LAVER and MESNIL, MUSGRAVE and CLEGG, all give long lists of drugs and chemicals used without avail. These we have retested, and have tried in addition sodium, potassium, and ammonium fluoride, fluorescene, chrysoidin, and various preparations of silver and mercury, especially the newer compounds. The results have coincided with the findings of other investigators. Up to March, 1904, the only drug of any value was arsenic; EHRLICH and SHIGA then published their results with a new dye named by them 'Trypanroth,' and these two preparations are the only ones found to be of value in the treatment of trypanosomiasis.

As animals have for a considerable period been known to be liable to infection with the various pathogenic trypanosomes, it was only natural to turn to the veterinary profession to see what drugs have in their hands proved of service in the treatment of these diseases. Of the countless drugs tried arsenic is the only one which has given encouraging results. Cases are on record where cures have been reported, but, on the other hand, many cases which have been treated by some form of arsenic and which have improved as long as the administration of the drug was kept up have shown parasites when it was discontinued and have succumbed to the infection. BRUCE records cases in which large doses of sodium arseniate were given, and so long as the treatment was continued parasites were hardly ever found in the blood, the animal retained its strength and weight and was able to work, but on the treatment being discontinued the parasites quickly reappeared and the animal began to fail. MOORE and CHICHESTER report the effect of the intravenous inoculation of cattle, after dosage the animals regained their weight, the yield of milk which had diminished became normal and the parasites disappeared, whilst animals which had not been treated quickly died. We have, therefore, evidence that arsenic is of use in prolonging the life of the animal. LAVERAN, in 1902, reported his results in treating rats infected with the Nagana parasites. The life of the animal was prolonged, the parasites disappeared, and the animal seemed to have recovered. When treatment was

discontinued after a varying length of time the parasites reappeared, the disease pursued its normal course, and the majority of the animals died. Some few recovered, but the animals were not rendered immune, as LAVERAN proved by reinoculation.

He had, however, been able to keep infected rats alive for periods many times exceeding those of untreated animals. LAVERAN and MESNIL record very unfavourable toxic effects with the ordinary preparations of arsenic. At McGill and Liverpool, in our hands the experiments showed the same toxicity of the drug, the tendency to cause ulcers and extensive sloughing despite all asceptic precautions. The results obtained as to the prolongation of the animal's life was the same. On the arrival of natives suffering from Trypanosomiasis it was felt that some form of arsenic treatment was indicated. Subcutaneous inoculations of sodium arseniate caused so much pain that this form of medication was abandoned. BRODEN had reported the favourable results obtained by the administration of arsenic in the form of *Liquor Fowleri*. Resource was made to this form of the drug, but the surviving natives were sent back to the Congo before the effects of the different preparations could be determined.

Animals infected with the various pathogenic trypanosomes were subjected to treatment; it was found that with sodium arseniate, the most useful form of arsenic, sloughing was prone to occur. In such a case the animal very often became so depleted by the ulceration that it succumbed to a secondary affection. Moreover, the use of arsenic in the form of sodium arseniate is all too apt to cause toxic effects. The administration of the preparation may be continued for some time, favourable results occur and then, unfortunately, toxic symptoms appear. If the drug be discontinued on account of the untoward symptoms the parasites very quickly make their reappearance and the animal dies. Moreover, despite medication being continued, the parasites which have disappeared will reappear and continue to be present and even augment. In such cases an increased amount of arsenic will not affect the termination of the disease. We have, therefore, to realise that the ordinary arsenic compounds when administered only produce a temporary, favourable effect, that, if long continued, the animals will die either from the parasite or from the arsenic or from both. Hence, some other compound is indicated. It is for this reason that the newer compounds of arsenic have been experimented with in order to find a preparation capable of being used over a long period and in high doses without producing toxic symptoms. Of the various preparations tried, a meta-arsenic anilin compound, atoxyl, has proved the most satisfactory, but it is not ideal. It is not non-toxic as dogs, kittens, guinea-pigs, and rabbits have shewn toxic symptoms and succumbed, but it is not so toxic as sodium arseniate. It does not produce the sloughing which so often follows the subcutaneous or intravenous inoculation of sodium arseniate, it causes no pain, and its administration can be continued over a period of many months even when used in extremely high doses.

It is the only remedy at present giving any prospects of a cure. In the treatment of cases a rational method of treatment must be adopted. It is useless, for instance, to only administer arsensic for a short period or until the parasites have apparently disappeared from the peripheral circulation. The drug must be administered in as high doses as possible, and it must be continued even after all the favourable signs are present, viz., disappearance of parasites from the blood ; increase of weight, improvement in the blood count and percentage of haemoglobin ; loss of the autoagglutination phenomenon of the blood corpuscles ; decrease in and more regular temperature. From time to time susceptible animals ought to be inoculated with large quantities of the patient's blood at least in amounts of 5·0 to 15·0 c.c. At the same time all aids in building up the physical condition of the individual should be used. If such a *régime* be carried out, and treatment be begun at an early period, the prognosis (based on the experience of treated animals) will be good.

With animals, on the other hand, the question whether treatment with arsenic is indicated calls for some consideration as LAVERAN and MESNIL, BRUCE, and others have pointed out that there is some danger of treated animals becoming a source of infection, by filling the position in these diseases occupied by the native in malaria. The arsenical treatment will cause a temporary disappearance of the parasites from the blood. Some animals may entirely recover, but the majority suffer a relapse, and these latter are then a source of infection for the surrounding country.

On the publication of EHRLICH and SHIGA's results in the treatment by trypanroth of rats infected with *T. equinum*, some of the dye was procured and a series of comparison experiments was made. The results coincide with the findings of the other workers. It is not a very safe drug. Ulceration is very apt to occur, the sloughing is often extensive and persistent. The tendency to cause nephritis is marked and hence its use, when continued, with arsenic is often apt to lead to fatal results, especially in guinea-pigs. High doses of the dye must be employed, and on account of its chemiotoxic properties intramuscular administration is the best method. Very often animals injected with large quantities of the dye appear to be in a stuporose condition which may wear off or persist up to death. Shortly after the injection of trypanroth the tissues commence to turn a bright red colour, and the secretions, urine, etc., are stained. The staining persists for a very long time ; the internal organs may show the staining of the dye for eight weeks or more after injection. We cannot claim to have cured any animal infected with the parasites of Surra, Nagana, Dourine ; the disease, especially in rats and mice, may be greatly prolonged, but the animals eventually die. Larger animals, especially dogs, cats, and monkeys, frequently show toxic symptoms with small doses. On the other hand, a bitch injected subcutaneously with fifteen to twenty c.c. of one per cent. trypanroth two and three times a week showed very little toxic symptoms besides a somnolent condition which lasted for about eight hours after each injection. The combination of trypanroth and

arsenic has given better results both in the hands of LAVERAN and ourselves. The animals can live for a longer period; the administration need not be so often repeated. The drawback is that the kidneys quickly become affected. Animals infected with *T. dimorphon* do not seem to react well to arsenical treatment by itself; trypanroth medication only causes the parasites to temporarily disappear; the combination of arsenic and trypanroth causes the parasites to be absent for a longer period. Various methods have been tried. Administration of trypanroth and arsenic together, or the dye first, followed in twenty-four to thirty-six hours by arsenic or *vice versâ*. It is the latter treatment which has given most satisfaction in our hands. LAVERAN administers high doses of arsenic and follows twenty-four hours later with an intramuscular injection of trypanroth.

Arsenic has been tried in combination with the other dyes but without results.

Arsenic and human serum in the treatment of rats infected with Nagana and Surra have prolonged the life of the animals. A guinea-pig inoculated with *T. Brucei* did not seem to improve, the serum was given in amounts of ten c.c. once a week, but no more serum being obtainable the treatment had to be stopped, and at the end of three weeks the parasites then reappeared. Animals infected with *T. dimorphon* do not show as marked a reaction to serum treatment as do Nagana, Surra, etc.; the employment of arsenic and human serum does not appear to promise well. Human serum and arsenic combined has been tried on animals infected with *T. gambiense*; the control animals treated with arsenic alone showed as much, if not more, improvement as those with the combined treatment.

Arsenic and Baboon serum; very little serum was obtainable, three mice infected with *T. gambiense*, *T. dimorphon*, and *T. Brucei*, respectively, were used. The results were indefinite.

Other sera including horse, cow, sheep, goat, dog, cat, rabbit, and guinea-pig have been tried. The sera were produced from both healthy as well as from infected animals which had the disease in a chronic form or were apparently cured. The sera were used alone or in combination with arsenic, with disappointing results, for in no case could a definite and permanent decrease be ascribed to the action of the sera. Polyvirulent sera have also been tried without success.

ATOXYL EXPERIMENTS

The trypanosomes and animals used for carrying out the investigation were as follows :—

T. gambiense, in monkeys, dogs, puppies, kittens, rabbits, guinea-pigs, rats, and mice.
T. Evansi, in horse, dogs, rabbits, guinea-pigs, rats, and mice.
T. Brucei, in cow, rabbits, guinea-pigs, rats, and mice.
T. equinum, in dogs, rabbits, guinea-pigs, rats, and mice.
T. equiperdum, in pups.
T. dimorphon, in dogs, pups, rabbits, guinea-pigs, rats, and mice.

The drug was used only upon animals showing the effects of the parasites, such as loss of weight, anaemia, fever, and autoagglutination of the corpuscles, and no animal was used until its blood contained numerous parasites. The numbers of the parasites present differed according to the species of animal and the disease. In the majority of the experiments control animals which were not treated and had been inoculated at the same time as the treated animals were used. In all cases the control animals died.

Intravenous inoculation was used only on rabbits, all other animals were injected subcutaneously. Treatment was continued for one to three months or until increase of weight, diminution of the anaemia and entire absence of parasites from the blood, as far as microscopical examination could determine, was noted. At various periods susceptible animals were inoculated with the blood from a treated animal. When treatment had been discontinued for one to three months or longer, the animal was bled or killed and all the blood available was used to inject susceptible animals. Inoculated animals whose blood has given negative results after three to six months or after longer periods have been inoculated with virulent blood and have taken the disease, thereby showing that no immunity was conferred by the previous inoculation.

T. gambiense.—Rabbit, ♂, weight, 2,010 grammes. Parasites appeared on the twelfth day. On the forty-sixth day, numerous trypanosomes were present ; it had lost weight (1890 grammes). A blood count gave reds, 4,980,000 ; whites, 8,860 ; haemoglobin, sixty-seven per cent. For three-and-a-quarter months it received 1·0 c.c. of five per cent. solution atoxyl three times a week, gradually increasing the amount to 1·0 c.c. of ten per cent. solution. It then weighed 2,000 grammes. The blood count was : reds, 6,640,000 ; whites, 6,200 ; haemoglobin, eighty-eight per cent. The blood in quantities of ten c.c. was non-infective. The autoagglutination of the corpuscles was lost. Thirty-two days later it was very ill ; it was therefore bled to death, and the whole of its blood injected into a monkey. This monkey has never become infected. The *post-mortem* showed severe haemorrhagic cystitis, the bladder in parts being almost gangrenous and acute septic peritonitis, especially around the bladder. The spleen showed no congestion, but the connective tissue was slightly increased. The kidneys and liver were normal.

Rabbit, 889, inoculated October 26 ; weight, 1,760 grammes. Blood count : reds, 6,620,000 ; whites, 6,700 ; haemoglobin, eighty-nine per cent. Parasites were seen from November 8 up to January 10 ; the trypanosomes were always present, but in small numbers ; they then increased to eighteen to twenty to a field. The anaemia was pronounced, and loss of weight was noted. It then weighed 1,540 grammes. Blood count : reds, 3,880,000 ; whites, 11,800 ; haemoglobin, sixty-three per cent. It could hardly sit up, and remained most of the time lying down. This animal was given 0·8 c.c. of five per cent. solution atoxyl. At the end of eighteen hours the parasites were absent from the blood. Doses were given twice a

week, beginning with 0·5 c.c., and increasing to 2·0 c.c. of a five per cent. solution. The blood in large doses is non-infective. The animal is vivacious ; the coat is smooth and thick. Average weight, sixteen hundred grammes. Several guinea-pigs infected for about two months, and showing twenty to forty parasites to a field, have been treated. They were injected subcutaneously with 0·3 c.c. of ten per cent. solution. At the end of the fifteenth hour no parasites were seen. Treatment was 0·1 to 0·3 c.c. of five per cent. solution three times a week for two months. The animals all increased in weight. One died sixty-two days after treatment was discontinued. Three rats inoculated with its blood never became infected. The second and third pigs were killed at the end of eighty and one hundred days after stopping treatment, and the blood used to inoculate controls. No control has shown the parasites. Virulent strain. *Rhesus.*—Weight, 2,815 grammes. Inoculated November 16. Blood count: reds, 5,220,000; whites, 12,800; haemoglobin, seventy-eight per cent. On November 31 parasites were seen. Two days later, twelve to seventeen to a field were noted. The weight was 2,420 grammes. Blood count: reds, 4,600,000; whites, 7,200 ; haemoglobin, seventy-three per cent. The parasites counted per c.mm. gave 100,000. Oedema of the eyelids and bridge of nose was present. The animal was given 0·8 c.c. of ten per cent. solution atoxyl subcutaneously. Four hours later : reds, 4,450,000 ; whites, 24,800 ; parasites, 40,000 per c.mm. At the eighth hour : reds, 4,740,000 ; whites, 25,200 ; parasites, one to eighty-nine fields. Between the fourth and eighth hours the parasites were seen to become remarkably degenerated and deformed ; many phagocytes were present. At the twenty-fourth hour after injection a count gave reds, 5,050,000 ; whites, 46,000. The blood was negative. The leucocytes remained high for a couple of days, and then fell ; in none of the phagocytes could any remains of trypanosomes be found. Treatment, 1·0 c.c. of ten per cent. solution was given twice a week. The animal increased in weight. The autoagglutination of the corpuscles began to be less accentuated, and the number of erythrocytes and the haemoglobin rose. The local oedema disappeared. On the thirty-ninth day dysentery appeared, and the animal succumbed on the forty-eighth day after injection. The autopsy showed a very severe haemorrhagic and necrotic enteritis, with slightly enlarged spleen. Kidneys normal. Glands small ; inguinal group haemorrhagic. The blood was non-infective in amounts of 1·0 c.c., but infective if fifteen c.c. of pure blood was used. Unfortunately, the arsenic was discontinued on the appearance of dysentery. A second monkey, inoculated from the first *rhesus* just before treatment was begun, was treated with the same doses of arsenic ; the parasites disappeared in the same way, but the animal quickly succumbed to dysentery.

Many rabbits inoculated with this strain have been treated. It was found that unless treatment was started early that the majority of animals died as it was so exceedingly virulent. With these animals treatment was begun earlier and higher

doses given than with the standard 'Gunjur' strain. Despite treating the animals early some died. With this strain treatment had to be kept up longer. Some rabbits have survived eight months after injection, while all the controls have died in fourteen to thirty-six days. Guinea-pigs infected with this strain do not react so well to the treatment. Rats must be treated early and with high doses if treatment is to be successful. Mice infected with this strain react if treatment is commenced early enough.

T. brucei.—Guinea-pigs inoculated for two months. Average loss of weight one hundred and fifty to one hundred and ninety grammes. Parasites forty to sixty to a field. Initial dose, 0·4 c.c. five per cent. solution ; parasites absent in about eighteen hours. Treatment, two to three times a week, 0·1 of five per cent. solution. At end of two to three months treatment was discontinued ; the animals had increased in weight. At periods, one to two-and-a-half months after stoppage of arsenic, the animals were killed and their whole blood used to inoculate rats. None of the rats inoculated from these guinea-pigs have ever shown parasites. Some of the control rats inoculated during the first five weeks of the treatment have become infected after lengthy incubation periods. These treated guinea-pigs have shown increase of weight and lessening of the agglutination of the blood cells. Non-treated guinea-pigs have lived on an average forty to forty-five days. One treated pig had lived nine-and-three-quarter months since becoming infected, or five-and-a-quarter months since discontinuation of treatment.

Rats.—Treatment was begun two to three and four days after parasites appeared, when the animals were in the last stage of the disease and showed the semi-comatose condition which is so often met with. Rats of one hundred and twenty-five grammes in this last stage, and showing two hundred or more parasites to a field, have been given 0·5 c.c. of five per cent. solution. The parasites have been absent from the tail blood at the nineteenth hour. If treated twice a week with 0·3 of five per cent. solution, and the amount gradually increased, the parasites remain absent, the blood is non-infective and the animal puts on weight. Some have lived one hundred and twenty-six days from date of inoculation, and fifty-seven days after treatment was stopped ; they have succumbed to broncho-pneumonia or dysentery. The subinoculated animals have remained uninfected. Rats treated only once with 0·5 c.c. of five per cent. solution show parasites in their blood again in six to eleven days, and unless treatment be recommenced, the disease will pursue its natural course.

Cow.—This animal was inoculated subcutaneously from a guinea-pig. Parasites appeared on the sixth day but were very scanty. A severe enteritis occurred on the ninth day, but the animal survived the attack. The parasites slowly increased. Loss of weight and marked anaemia developed. Parasites continued to increase, the animal was then given 1·5 grammes of atoxyl in ten per cent. solution subcutaneously. The parasites disappeared slowly. Leucocyte count increased. Deformed degenerated

trypanosomes were seen at the end of a day. The parasites were absent from the blood; 2·0 grammes of atoxyl was given twice a week. The animal was very anaemic, emaciated, and weak. It improved for a time. Unfortunately, our early departure prevented us making a study of this case, and, moreover, as there was no data as to the dose of the drug the administration had to be cautiously proceeded with.

T. evansi.—The results in the small laboratory animals are about the same as with Nagana. The animals have lived as long.

A horse was injected subcutaneously with blood from an infected rat; many divisional forms seen. Incubation four days; temperature rose on fifth day and parasites rapidly increased in numbers; the animal eat little and stood with head hanging down. The numbers increased to forty thousand to the c.mm.; the haemoglobin fell from ninety-five to sixty-three per cent.; no great change was noted in the red and white blood cell count. When definite loss of weight and anaemia had occurred and the parasites were about five hundred to the cover, 1·0 gramme of atoxyl in the form of a ten per cent. solution was given subcutaneously; in thirty hours the parasites had disappeared. A slight leucocytosis was observed; 2·0 grammes were then given and this was continued twice a week. The animal grew fatter and the blood count improved.

T. equinum.—Guinea-pigs react to treatment quickly and for a considerable time, but there is a tendency for some of them to show parasites in their blood one and two months after treatment has stopped. On the other hand, one treated for two-and-a-quarter months survived eight-and-a-half months after inoculation, treatment being stopped for two-and-three-quarter months. This animal was killed, and rats and guinea-pigs inoculated with its blood have never become infected. A rabbit which had been infected for twenty-eight days showed the loss of hair around the eyes and nose, and the purulent discharge from the eyes, nose, and urethra. The oedematous condition of the ears and genitals has by treatment with 1·0 c.c. of ten per cent. solution once every five days disappeared so far that all the external signs are wanting and no parasites are found; it has, up to date, lived seventy-six days.

T. equiperdum.—The work has been entirely on pups; these parasites have reacted in the ordinary manner, but the tendency of young puppies to manifest toxic symptoms has resulted in the experiments only prolonging the disease for one to two weeks longer than their controls. A bitch, inoculated January 14; positive January 20; large number first appeared on the 26th, temperature markedly irregular. Periodicity noticed; usually parasites absent for one to three days, then a rapid increase. Animal became very anaemic and thin. The blood count before inoculation gave 5,860,000 reds; 11,000 whites; and ninety-seven per cent. haemoglobin. On February 25 the reds were 3,780,000; whites, 14,000; haemoglobin, sixty-five per cent.; parasites were six to eight to a field. Trypanroth sixteen c.c. one per cent. solution administered. In thirty-six hours no parasites

were seen, but they reappeared six days later. Ten c.c. of one per cent. trypanroth was then given every eighth day ; the temperatute lowered. The parasites decreased but the anaemia persisted ; March 19, atoxyl 1·5 c.c. of ten per cent. solution subcutaneously given ; parasites disappeared to reappear eight days later. Despite large amounts of trypanroth the animal succumbed to the disease. A blood count, March 30, reds, 1,710,000 ; whites, 6,600 ; haemoglobin, forty per cent. ; trypanosomes, 24,000 per c.m.

T. dimorphon.—This parasite has proved harder to combat with atoxyl or any other form of arsenic than the preceding parasites. LAVERAN also notes this peculiarity. Guinea-pigs showing a severe infection react extremely badly, they appear to manifest a greater tendency to toxicity than do similar animals infected with Nagana or other trypanosomes. The disappearance of the parasites is often less rapid, sometimes they never absolutely disappear, an occasional one being seen in the preparation. In the endeavour to cause the typical reaction a fatal dose may easily be given. One guinea-pig out of twenty-two experimented with survived three months. This animal was only treated once a week with 0·3 c.c. of five per cent. solution. It improved considerably, but towards the last the parasites reappeared, and the animal succumbed. The autoagglutination of the blood-cells never completely disappeared ; the animal always exhibited some fever.

Rabbits.—React better to the drug, but the effect is more transient than with other trypanosomes. Higher doses have to be given. The life of the animal can be greatly prolonged, and for one or two months the general condition is improved. After that time parasites once more reappear ; by increased dosage they may again disappear. Towards the end larger doses have to be given than at first to produce the effect on the parasites. Here it may be noted as applying to all species of animals infected with *T. dimorphon* that the parasite in animals treated for some time will hardly be recognizable ; it presents a deformed appearance, its flagellated extremity is shortened so that scarcely any movement occurs. Blood from such an animal will be declared negative unless each field is gone over thoroughly. All peculiar bodies present must be examined, and over any such object minutes spent to determine if there is any movement. This point seems rather superfluous, but our experience is that the ordinary careful observer usually fails to find these degenerated, deformed parasites, and it is probably for this reason why so many negative diagnoses are made. Dogs and cats infected with this parasite show only a slight retardation of the disease ; the numbers are greatly reduced or absent, but tend quickly to reappear.

TRYPANRED

This dye has been mostly used in connection with animals infected with *T. equinum.* The results tally with those of EHRLICH and SHIGA, and the later report of LAVERAN and MESNIL. Some rats injected with this compound have lived as long as one

hundred and seven days; in one case sixty-three days without any treatment being given, this rat died from broncho-pneumonia, its blood was non-infective. Mice have also shown the favourable results of this treatment, but they do not appear to be able to withstand large doses.[1] Rabbits and guinea-pigs inoculated with this substance have had their lives prolonged for a considerable time, the parasites disappear completely, and if the administration of the dye be continued the trypanosomes may not be seen for thirty to sixty days; but the animal does not show the favourable reaction which attends the administration of arsenic, the anaemia is very little lessened, very often it is increased, the weight is not recovered, the temperature remains irregular. Very often if high doses are given toxic symptoms appear, these are characterized by more or less somnolence, the animals always appearing 'heavy,' there may be a slight discharge from the eyes. The face is often puffy, the urine in such cases usually has albumen; after a time these symptoms wear off. They are especially marked in rabbits after intravenous injections. Rabbits can withstand intravenous injections of 1·0 to 2·0 c.c. of one per cent. solution, and it appears as if the administration through a vein gives better temporary results, as the parasites, even *T. dimorphon*, disappear more quickly than by the subcutaneous method. The disadvantage is that the anaemia appears more pronounced, and the toxic symptoms severer. No rabbit or guinea-pig has been definitely cured.

Dogs and cats react fairly well to this dye, the life of the animal is prolonged, but no cure has been affected. Towards the last, despite repeated injections of this dye, the parasites continue to increase, the animal becomes a living skeleton, and usually develops a purulent discharge from the eyes and urethra.

T. evansi, *T. brucei*.—Rats and mice inoculated with either of these parasites, and injected with this dye, live longer than infected controls. The disappearance of the parasite is only temporary; after four to six days the parasites reappear, and though they can be caused to disappear once or twice they sooner or later reappear, and the animal dies, sometimes at a time when only very few parasites are present in the blood. With Nagana and Surra, inoculated animals which have been treated have not lived over thirty days, the majority dying in fifteen to nineteen days.

Rabbits and guinea-pigs react for a while, but the action on the parasites is not so marked as with Caderas infected animals.

Dogs and Cats.—The same results as with rabbits and guinea-pigs, only a very short prolongation of the disease is observable.

T. equiperdum.—The duration of the disease is only slightly affected by this dye. If injected early in the disease when the pup shows hardly any parasites a considerable prolongation of the animal's life occurs, but once large numbers of

1. Extreme care has to be exercised in administering this solution. With rats and mice the preferable method seems to be to inject into the thick muscles of the hind legs. The hair is shaved off and the part disinfected before the injection. Guinea-pigs and rabbits ought to be treated in the same way. Dogs and cats can be injected in the deep muscles of both fore and hind limbs, in the back, or in the loose tissue of the neck. Monkeys are difficult as the skin is extremely sensitive, but the thighs offer the best site for inoculation.

parasites are present in the peripheral blood very little benefit occurs from a continuance of this treatment.

T. dimorphon.—The dye exerts a slight influence on this parasite, the numbers in the blood diminish, and many deformed ones are seen after a few days ; the organisms increase in numbers. In a few instances the parasites are absent from the peripheral circulation for a short time. Dogs show only transient improvement with a diminution of the parasites followed by a rapid increase in their numbers ; this phase lasts a few days only. Rats and mice show a temporary reaction, their lives may be prolonged for fourteen days ; at times the treated ones appear to succumb more quickly than the non-treated controls.

Rabbits and Guinea-Pigs.—The same temporary benefits are derived from injection of this dye.

Testing the action of the dye on the parasite by the addition of some of the solution to defibrinated blood containing trypanosomes, shows that with all of the different pathogenic trypanosomes a slight microbicidal action is manifested. The contact must be for some hours before the action will be noted ; it is, however, a definite action as control tubes show. EHRLICH and SHIGA record the preliminary injection of animals with trypanroth, and after a few days injecting the animal with some virulent blood. They believe that the dye forms anti-bodies. A goat was therefore regularly injected with the dye, and after some weeks some of the blood was drawn off; at first it was noted that the action of the serum did not differ from normal goat serum. As the animal got more under the influence of the dye it was noted that contact of living trypanosomes with this serum produced deleterious effects on the parasites which very often became deformed. If some of this serum was injected into mice with *T. equinum* a diminution in the number of the parasites was noted. Unfortunately, the injection of this dye for a long period caused the goat to become very run down, and the regular injection for a time had to be stopped. On the goat regaining its health it was once more given a course of injections with the dye. The serum had the same effects. In four mice the administration of this serum has prolonged the lives of the animals for thirty-one to forty-eight days. The serum is of a light, clear, fuchsin-red colour after five grammes have been given the goat. The amount of serum necessary is about 1·0 c.c. for a mouse of twenty-two grammes weight. As more of the dye is injected the amount of serum necessary is lessened. The experiments are too few in number to possess any practical worth. As the sodium arseniate and trypanroth had proved of use a combination of these two substances was tried for ten months.

Atoxyl-trypanroth combination has proved of benefit. With animals infected with *T. dimorphon* the results are more promising than in controls treated with either of these drugs separately. The parasites disappear rapidly, remain absent if the administration of the arsenic and the dye is pushed ; the animal increases in weight ;

the anaemia lessens, and the autoagglutination disappears. As mentioned before, trypanroth caused a nephritis, therefore, the combination of these two subjects is fraught with difficulties. In order to obtain results both drugs have to be pushed. It therefore often happens that the animal acquires a severe nephritis and succumbs. The effects of this combination have been noticed on other trypansomes.

The combined treatment offers a promising mode of medication, providing the untoward effect can be diminished or abolished. Very many of the smaller animals, especially guinea-pigs, have died from the effects produced by trypanroth.

Action of Atoxyl and Trypanred on the Trypanosome

The action of atoxyl on the various trypanosomes has been studied, and after numerous observations, continued for the whole period during which the drug was administered, the effect appears to be as follows :—

On administration of arsenic compounds into an animal showing numerous parasites in the blood the following action on the trypansomes will be noticed. For the first three-and-a-half to four hours, depending on the dose used, very little change in the parasites can be noticed. Between the fourth and fifth hour the effect on the trypanosomes is evident. Some parasites appear to be swollen and their movement is less rapid. If now a series of blood specimens be examined at intervals of twenty to thirty minutes the following changes will be seen. The number of slowly moving trypanosomes increases, many parasites will be seen to be almost motionless. The protoplasm takes on a peculiar ground-glass appearance, and dark granules appear in the protoplasm ; very often a whole series of granules one behind the other, sometimes in pairs or all clumped together, are seen lying between the macronucleus and the anterior end or distributed through the whole body of the parasite. At the same time vacuoles are observed, oftentimes very large. The trypanosomes become deformed, assuming various shapes, the most common being a kite-shaped form with fairly long flagellum, and a tadpole-like one with hardly any free flagellum. These forms especially exhibit greatly impaired movements. At the same time a noticeable increase of the leucocytes is discernable ; phagocytes begin to appear, very often groups of five to seven will be seen. Up to this time (sixth to seventh hours) the trypanosomes, though decreased in numbers, are still present in considerable quantities. Suddenly in the course of an hour the numbers may drop from forty to two to three to a field or less ; coincident with this is a very marked increase in the number of leucocytes, especially phagocytes. From the ninth to the fourteenth and sixteenth hours the changes are less pronounced and rapid, the trypanosomes gradually disappear. At the eighteenth hour, provided the animal has been injected with the correct amount, the parasites are absent from the peripheral circulation and, even though the blood be centrifuged, none can be found.

My colleague, Dr. BREINL, and myself have observed a large number of experiments, examining the animals every one-and-a-half hours until the four-and-a half hour, then every half hour until the parasites were almost absent, and continuing the observations hourly until the twenty-fourth to thirty-second hour after starting the experiment. From a series of these observations, we have determined that in hardly any of the forty-six continuously observed animals were parasites to be found after the eighteenth hour. Should, however, the drug be given in smaller amounts the process takes longer, lasting from thirty-six to forty-eight hours.[1]

Phagocytosis has been observed on three occasions. We have witnessed the engulfing of a trypanosome still alive though almost motionless, and on other occasions the ingesting of dead trypanosomes.

Observations on the effect of *Trypanred* on the trypanosomes show that almost the same changes take place, though the process is somewhat slower, usually requiring forty-eight hours or more ; leucocytosis also appears to be very marked in animals treated with this drug.

LEUCOCYTOSIS

In the observations on the action of arsenic and trypanroth on the trypanosomes, it was noticed that a marked increase of leucocytes occurred. Phagocytosis having been seen, it was determined to try the effect of hyperleucocytic agents on the parasites. In the work on the effect of diphtheria antitoxine on trypanosome-infected animals a certain amount of leucocytosis had been observed, but it was not marked. Advantage has, therefore, been taken of such agents as nuclein (this also affects the kidneys). Thanks to Professor SHERRINGTON, we were able to try the effect of colchicine on the infected animals. This substance causes a leucocytosis, but it only produces an increase of some six thousand in thirteen hours. Nucleinic acid acts in the same way. We have been induced to try the effect of administering the atoxyl or trypanred, or the combination atoxyl-trypanred, and following this treatment with the hyperleucocytic agent. Unfortunately, no better effects have been observed. This line of work ought not to be neglected, as it is quite evident that the leucocytes play a rôle in the decrease of the parasites. Animals which have been carefully observed every day show from time to time a decrease in the number of parasites in the peripheral blood. If the decrease be sudden and marked a vast increase in the number of leucocytes is determinable.

THE ACTION OF BACTERIA UPON TRYPANOSOMES

It has been noted in animals suffering from trypanosomiasis that should a localized abscess occur a certain decrease in the number of trypanosomes in that region can be made out. In rats the tail from snipping or pressure may become ulcerated or

1. Blood films were made before beginning the experiment, and at every examination. The results of the examination of these stained films bear out the observations on the fresh blood films. The significance of the granules seen and the phagocytosis will be discussed in a fuller report.

gangrenous, in such a part very few parasites will be found although the blood above the area may be swarming. Moreover, the movements of the parasites are slow and show degeneration and deformity, whilst phagocytes will be found containing the remains of trypanosomes. Acting on these observations experiments have been made with various bacteria. Guinea-pigs suffering from tuberculosis seem to be somewhat more susceptible to trypanosome infection than healthy ones. Mice, already infected with trypanosomes, and then inoculated with *B. anthracis* and the *B. sui*, show at times a certain amount of retardation of the disease. The parasites do not appear to augment so rapidly. Ordinary cultures of pyogenic cocci are too virulent for the animal. If a very attenuated culture of *Staphylococcus pyogenes aureus* is inoculated with *T. dimorphon*, the animals show a marked diminution in the number of parasites, but they persist for some days. Unfortunately, the subcultures never act in the same way and show increase in the virulency. In one of some eight rats infected with *T. brucei* and then injected with *Leuconostoc mesenteroides* there was a lessening in the number of the parasites ; this animal was subsequently injected intraperitoneally with a culture of KLASSE's *Gonodiplococcus* of scarlet fever. The parasites disappeared, but the animal died one-and-a-half days later from a septic peritoneal infection due to the wounding of the gut. Cultures of *B. typhi*, *B. coli*, *B. diphtheriae*, so far as we have been able to observe, have proved of no avail.

In connection with these observations it is interesting to record the work of NISSLE ; unfortunately, only a review of his preliminary paper is available. He inoculated intraperitoneally a number of rats infected with the Nagana parasites with one-twentieth of a loopful of a *B. prodigiosus* culture grown on potato ; in twenty-four hours the trypanosomes disappeared, and some of the rats died from intoxication in half to three hours after injection. During the disappearance of the parasites he observed all kinds of endo-globular forms. It must be borne in mind that the injection of a culture causes a leucocytosis, and whether NISSLE's results depend only upon this or upon the toxic products of the bacteria remains to be seen. It is hoped that this question will not be neglected.

CONCLUSIONS

From the experimental work with various therapeutic agents the following conclusions can be made :—

1. That animals suffering from trypanosome infection react favourably to only a few agents, of which arsenic is the only drug which seems to exert a more than transient action.

2. That the greater the amount of arsenic introduced into the system of the animal the greater and more permanent the effect on the parasite.

3. That arsenic medication is indicated in the treatment of individuals suffering from Trypanosomiasis. That the treatment ought to be long continued and regularly

administered in as high doses as the case can stand. That all aids to building up the system should be employed.

4. That in trypanred we possess an agent of some use in the treatment of trypanosomiasis. That certain trypanosomic diseases appear to be more amenable to its action than others. That in the substance at present available there is need for improvement in order to abolish its toxic effects.

5. That a combination of arsenic and of an improved form of trypanred would seem indicated in the further investigation of the cure of trypanosomiasis.

6. That further efforts ought to be made to produce less toxic forms of arsenic suitable for injection.

7. That the action of both arsenic and trypanred causes a hyperleucocytosis and that this condition has an effect upon the parasites. In confirmation of this is to be recorded :—

(*a*) The hyperleucocytosis coincides with the time when the parasites suddenly disappear from the peripheral circulation.

(*b*) There is a smaller number of parasites present in necrotic, bacterially-infected areas than in the general circulation.

(*c*) The effect of certain bacteria on animals infected with trypanosomes.

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SUPPLEMENTARY NOTES

On some Animals still under Treatment on Drs. Thomas's and Breinl's Departure

Nagana cow (page 55), died eleven days later. The haemoglobin was eighty per cent., and the agglutination of the red blood corpuscles remained absent. Death due to extensive hydatids and pulmonary tuberculosis.

Surra horse (page 56), died over three weeks later. The haemoglobin which had at first risen under the atoxyl to eighty per cent. had fallen to sixty per cent. just before death. The agglutination of the red blood cells remained constantly present. In the intervals between the administration of the atoxyl, the parasites would return, and were twenty-five per field on the fourth day after the dose in the last week but one. Two doses per week were therefore commenced. This kept the number of parasites down, so that one per five fields was the highest reached. The animal, however, had become too reduced, and died three days after receiving the third dose at the smaller intervals.

Autopsy.—There was anaemia of all the organs, otherwise (spleen not excepted) there were no striking naked eye changes. Patechial haemorrhages were found in the muscles of the chine. There was a retropharyngeal abscess the size of a child's head communicating with the nasal sinuses, but it appeared to have been of some standing and not to have caused death. The animal was able to swallow readily up to its death.

Rabbit 839 (page 53), kept in perfect health for another month, and as the animal went off its food for a day or two the last dose given was only half the usual one. Two days later, however, the animal died, parasites having been found in the blood the day preceding, twenty per cover, degenerated and almost motionless from the effect of the atoxyl.

Caderas rabbit (page 56), still alive, September 28, 1905. In perfect health. No discharges of any kind; all hair regrown. No treatment since June 3. Blood negative since May 30 to microscopical examination. Its weight on April 25 was 1,770 grammes, and continued to fall till the end of May, when it was 1,190 grammes. Since then it has gradually increased to 1,795 grammes. It has now lived eight months. The head is usually kept over extended, which may be due to canker of the ears which was always present.

P. A. H. RADCLIFFE, M.B.

PART II. POST-MORTEM EXAMINATIONS WITH DESCRIPTION OF MACROSCOPIC AND MICROSCOPIC CHANGES

I. EXAMINATION OF ORGANS IN MAN (FOUR CASES)

A.—CASE OF THE NATIVE, KITAMBO

Death, June 12, 1904, eight p.m. ; *post-mortem* six hours later.

External appearances.—Body of middle height, very emaciated ; muscular development poor ; no exanthemata ; no oedema ; rigor mortis marked ; configuration of skull normal, markedly dolicho-cephalic. Conjunctivae and sclerae very pale and slightly jaundiced ; mucous membranes pale. The cervical and inguinal lymphatic glands were distinctly prominent. Thorax long ; intercostal spaces prominent ; abdomen retracted. External genitals normal. Superficial ulcers about three c.m. in diameter on the soles of the feet (probably produced by chiggers).

Brain and Spinal Cord.—The under surface of the scalp was very pale. Skull rather thin, the vessels on its inner surface occupied deep sulci. Circumference of skull fifty-two c.m. The dura mater adherent in places to the bone. The sinuses contained dark fluid blood and a few clots. The inner surface of the membrane was smooth and glossy. Cerebro-spinal fluid increased in quantity. The surface of the brain was of normal configuration ; the gyri appeared a little flattened. The superficial veins were very large, many were tortuous and they contained dark venous blood. The arteries were also engorged so that the whole surface of the brain was covered with a dark blue network, and greatly congested capillaries (Fig. 1). The leptomeninges were thickened, and in places whitish but not adherent to the brain. The pial vessels of the cerebellum were much congested and could be traced to the finest capillaries. The basal arteries showed no sclerosis. On section the brain substance showed irregularly distributed congested areas ; the basal ganglia showed puncta cruenta. The brain substance was doughy, soft, and very oedematous ; the ventricles were dilated ; the ependyma roughened ; the vessels very congested. The pons and medulla showed no macroscopic changes except congestion and some small haemorrhages about the size of pin heads. In the vertebral canal was a fair quantity of cerebro-spinal fluid. The inner surface of the spinal dura mater was smooth and glossy ; the pial vessels were enlarged and filled with dark blood ; the congestion was not equally distributed, being more pronounced in parts ; in others the vessels were only moderately filled. The cauda equina presented a striking appearance ; it was surrounded by a gelatinous tissue and its nerves were sheathed in networks of dilated vessels ; on section the congested vessels were seen as dark

red stripes entering the substance. In the region of the third dorsal segment, at the base of the right posterior horns, were four haemorrhages, the largest being about two mm. in diameter, and extending through two segments of the grey substance. The structure of the cord in the region of the haemorrhages appeared obliterated.

Thorax.—Diaphragm reached to the level of the fifth rib on each side. Both lungs were adherent to the diaphragm, but were free elsewhere ; parietal pleura normal.

Right Lung.—Pleura of the upper lobe normal ; that of the lower lobe showed dark blood coloured patches. The tissue of upper and middle lobes normal ; the lower lobe was congested and of a bright red colour.

Left Lung.—Under the pleura were numerous small haemorrhages. The lung tissue contained patches of congestion. The mucous membrane of the trachea was pale and covered with a small amount of clear viscid mucus. The thyroid gland normal. The nasal cavity and the sinuses in connection with it, as well as the tympanum, appeared normal. The pericardium contained a few c.c. of yellow tinged clear fluid.

Heart.—Size normal ; substance very pale and flabby. The chambers contained dark fluid blood and clots. The endocardium, the valves, and the intima of the aorta appeared normal.

Abdomen.—The cavity contained about two hundred and fifty c.c. of a light straw-coloured fluid.

Liver.—Of normal size ; its capsule here and there slightly thickened ; the parenchyma pale and friable.

The Gall Bladder.—Filled with thick, fluid, greenish bile.

The Spleen.—Enlarged (20 × 10 × 6 c.m.), the capsule very thick and partially adherent to the parietal peritoneum ; the pulp very dark purple-red and slightly diffluent ; on section, enlarged malpighian bodies could be seen in some parts, whilst in others no structure could be made out in the dark purplish mass.

The Kidneys.—Were of normal size, capsules stripped easily. Externally they retained the deep embryonic furrowings ; the cortex presented an appearance of fatty striation, otherwise they were normal.

The Bladder.—Contained nearly half a litre of cloudy urine. The mucous membrane was pale. The genital organs showed no changes. The suprarenals were greatly congested. Pancreas normal.

Stomach and Intestines.—The mucous membrane of the stomach was pale, that of the small intestine somewhat congested ; the large intestine being normal. The stomach contained a little mucus ; the small intestine was partially filled with yellowish-brown chyme, and here and there in the recesses of its folds a few anchylostomes were found.

Lymphatic Glands.—Mesenteric lymphatic glands were enlarged and showed haemorrhagic infiltration, others were normal. The inguinal glands were greatly

enlarged, being six c.m. long and four c.m. broad. On cross section they appeared uniformly yellowish in colour.

Pieces were taken from all the organs and fixed in the usual fixing fluids :— ZENKER's, FLEMMING's, hydrarg. perchlor., and alcohol. The brain and spinal cord were fixed in four per cent. formalin.

MICROSCOPICAL EXAMINATION

The heart showed a fairly extensive small-celled infiltration in the endo-, epi-, and myocardium. The epicardial fat was well defined from the muscle, and in it was an accumulation of lymphocytes, large mononuclear and polymorphonuclear leucocytes. The infiltration extended along the connective tissue between the muscle fibres, and areas of infiltration were seen around some of the larger vessels in this situation, with a few mast cells and giant cells (these latter containing five to eight centrally-placed nuclei, or sometimes only fragments of nuclei). Here and there were large haemorrhages which extended from the perivascular connective tissue into the adjacent muscle, often destroying it. Frequently one could see, lying near a vessel wall, wedge-shaped patches of red blood corpuscles which were certainly the product of diapedesis, although no corpuscles were found in the wall itself. Many of the muscle fibres were smaller than normal, the striation was faint and around the nuclei were collections of reddish-brown pigment. These changes were for the most part equally distributed throughout the whole thickness of the myocardium, but in places were more marked in the left ventricle than in the right.

For the detection of bacteria, sections were stained by LÖFFLER's methylene blue, carbol fuchsin (Pfeiffer), carbol thionin and by GRAM's method. Only a very few bacilli (decolourized by GRAM's fluid) were found. These were considered to represent *post-mortem* contamination.

Such parts of the lungs as appeared normal to the naked eye were found on microscopic examination to be hyperaemic. The congested vessels often contained a good number of white blood cells. The bronchi contained desquamated epithelium and exudation. The lymph tissue around the bronchi showed hyperplasia. The pleura showed various-sized extravasations of blood in its connective tissues, the blood vessels being very large. The pneumonic-looking parts showed typical catarrhal pneumonia, the alveoli were filled with red blood and exudation cells. The process had gone on further in some parts ; there the whole tissue formed a homogenous mass interspersed with red blood corpuscles and exudation cells. In the consolidated parts were a good number of cocci staining by GRAM, and numerous GRAM-negative bacilli and cocci.

The sections of the *liver* showed a thickening of the capsule. Between the chains of liver cells were many partly degenerated blood cells, decreasing in number as the centre of the liver was approached. The connective tissue was increased in amount

and contained a few lymphocytes. Many of the liver cells had no nucleus, the protoplasm was vacuoli., and in various stages of fatty degeneration. A few of the cells contained small clumps of yellowish pigment occupying more than half of the cell. The blood vessels which were very much congested, contained a limited number of white blood corpuscles.

The *kidneys* presented the picture of parenchymatous degeneration. The protoplasm of the epithelial cells showed granular degeneration. The blood vessels, especially in the cortical region, contained very many white blood cells, and here and there in close proximity to the vessel wall were numerous red blood corpuscles. In places only scanty remains of normal kidney tissue were visible, the field being occupied by well stained connective tissue, its meshes entirely filled with red blood corpuscles. The glomeruli showed the same congestion, distended capillaries sometimes forming more than two-thirds of the whole glomerulus.

The *spleen* in section showed a thickening of its capsule. The malpighian bodies were few in number and not prominent, and from them irregularly defined processes ran out into the surrounding tissue. There were two zones to be made out in a malpighan body, viz., an external zone consisting only of lymphocytes, and a central one consisting of large cells containing a vesicular nucleus. Amongst the latter were large non-nucleated cells (forty to fifty μ in diameter), the protoplasm of which stained a dull red with eosine, and in addition a few leucocytes with eosinophile granules. The trabeculae were hyperplastic, and the vessels showed endothelial proliferation. The congestion of the spleen was most striking, especially in the periphery. Interspersed in the tissue were red blood corpuscles, sometimes clumped together in dense irregular heaps, in which a few lymphocytes were also included. The red cells showed indistinct contours, and by their staining reaction were evidently degenerated. Here and there were small irregular necrotic areas, in which only a few connective tissue cells were present. The cellular elements of the organ included very numerous large hyaline cells, containing inclusions of red and white cells, a very few nucleated red cells in the larger vessels, and, in the tissue of the pulp, giant cells with numerous irregularly placed and irregularly shaped nuclei and a large protoplasmic body. Throughout the spleen are found mono- and polynuclear leucocytes with eosinophile granulation. Iron-containing pigment is occasionally seen, both intracellular and free. In the vessels and in the tissue itself occasional filaria embryos were met with.

Lymphatic Glands.—The most marked changes were found in the lymphatic glands. The vessels were found to be highly congested in accordance with the naked eye appearance. Side by side with normal glands, in nearly all groups, there were others which contained very numerous red corpuscles, lying free in sinus-like spaces partially filled with large phagocytic cells. In nearly all the lymphatic groups were also transitional forms between normal glands and those showing the above described changes. The changes bore no proportion to the size. In the same lymphatic gland

often one part would be quite normal, while another part showed typical s... formation.

These enlarged glands in the first stage of their formation showed a thicke... capsule, with bands of fairly thick connective tissue running in from it towards ... centre and containing enlarged vessels showing distinct proliferation of their end... thelium. In some places the lymphoid tissue does not reach right up to the caps... but a lightly stained fine connective tissue meshwork intervenes, which contai... besides a few lymphocytes, a number of red corpuscles, large phagocytes, and in... containing pigment granules. The nucleus of the phagocytes is peripherally place... and their inclusions consist of the following:—numerous darkly-stained nuclei, ... size of lymphocytes, and red blood cells which either stain yellow with Van Gieson's method or appear merely as unstained vacuolar areas in varying number often filling the whole cell. The intervening spaces in the sinus are filled with coagulated fluid. Sinuses with the same contents as the peripheral one are found in very small numbers in the interior of the gland, recognizable at the side of the connective tissue strands by their light staining. The follicles are not so distinct as normally; they are large and not sharply defined from the surrounding lymphoid tissue.

The majority of the smaller glands presented a similar microscopic appearance: frequently there were a good number of red corpuscles between the lymph cells, and the sinus system was more or less pronounced. Sometimes there occurred only a short peripheral sinus, with one or two central off-shoots; in other places, the whole gland was interlaced by them. In some the follicles were normal, in others lightly stained, and contained, in addition to larger vacuolated cells and lymphocytes, a fair number of small cells two-thirds the diameter of a red blood corpuscle, which had an eccentric, deeply-stained, small nucleus and protoplasm staining faint pink with eosin. Besides, there were very many small nuclei both free and contained in other cells, staining darkly with haematoxylin. The blood vessels were much dilated, and contained, besides lymphocytes, a good number, as a rule, of large mononuclear and polymorphonuclear leucocytes and a few eosinophile cells.

Some of the larger lymph glands showed the change in a more advanced stage (see Fig. 6). In these, by a marked hyperplasia of the connective tissue, the lobules of lymphoid tissue became contained in a framework of connective tissue, producing thereby an alveolar structure. In the centre of the gland, an accumulation of connective tissue was extended by radiating septa to the capsule; this was thin, and had large dilated vessels surrounded by numerous lymphocytes running in it. There were no fat cells in the connective tissue of the glands, showing that these were true lymphatic glands, and not new formations in the adipose tissue. The sinus formation was as described in the first stage. Around the congested vessels were nearly always deposits of red corpuscles with a little blood pigment. Follicles were not to be seen.

Fig. - shows this transformation in one of the mesenteric glands. A sinus is

seen not far from the capsule, filled with lymphocytes, and surrounded by red blood corpuscles, these latter being easily distinguishable from the blood vessels by the absence of endothelial cells.

The cellular elements included divisional forms of lymphocytes ; giant cells, resembling those of bone marrow ; large hyaline cells with included red corpuscles in all stages of degeneration, sometimes only appearing as vacuoles ; mono- and polymorphonuclear leucocytes with eosinophile granulation ; phagocytes ; and plasma cells.

In some of the glands were seen all stages between phagocytes containing red cells and eosinophile cells. In the first stage were phagocytes with their nucleus placed peripherally, and the protoplasm of the cell filled with red corpuscles, stained a peculiar colour with eosine. The eosine-stained red corpuscles became smaller and smaller in their including cells, whose protoplasm in a later stage seemed to be unevenly stained a brilliant red tinge. Later, the nucleus changes its form and becomes polymorphonuclear, and in other cells one can almost see the eosinophile granules crystalizing out from this red-stained protoplasm. Some of the eosinophiles are destroyed in the lymph glands by phagocytosis, whilst others appear to enter the circulation. New formation of lymphatic tissue was noted ; for example, in the adipose tissue around the axillary glands there was an accumulation of lymph cells between the fat cells, and in some places there was distinct follicle-like formation around the vessels.

The *Bone Marrow* of the femur in some places had normal appearance of fat marrow, but in others however the marrow was very cellular, the fat tissue being reduced to a few islets, and replaced by cellular tissue composed of normal marrow cells with very few nucleated red corpuscles, and a large number of eosinophile cells. The vessels were greatly congested and there were frequently haemorrhages around the vessel walls ; the blood corpuscles showing degeneration. In different parts there were varying numbers of phagocytes containing red blood cells. Blood pigment was only infrequently met with. Here and there the whole of the tissue was replaced by a homogenous substance containing very few cells, staining yellowish with VAN GIESON and red with eosine, and presenting the appearances of gelatinous degeneration.

Brain and spinal cord were fixed in four per cent. formalin and hardened in alcohol and sections were cut from the different regions. The sections were stained by haematoxylin, eosin and VAN GIESON, WEIGERT'S method, WEIGERT-MARCHI, NISSL and UNNA, and for bacteria with LÖFFLER'S methylene blue, carbolfuchsin, PFEIFFER, carbol thionin and GRAM.

The pia and arachnoid shewed a fair amount of pigmented cells, similar to those observable in skin, the pigment being dark-brown and granular. The large and small vessels were surrounded in a varying degree by small-celled infiltration ; sometimes the larger vessels had a quite normal wall. The small-celled infiltration consisted of

lymphocytes, phagocytes, with all kinds of inclusions, also a few larger mononuclear and polymorphonuclear leucocytes, and a large number of red blood corpuscles. The same infiltration was found about the cells of the pia and accompanying it into the cerebral sulci and to a certain extent into the brain cortex itself. In the brain the infiltration occurred chiefly around the vessels of the deeper parts of the brain, those of the cortex showing relatively slight changes.

The vessels in the deeper parts were very large and congested, the perivascular lymph space dilated, sometimes empty, at other times occupied by coagulated exudation or filled with lymphocytes. The proliferated endothelium projected into the lumen. The vessels in the more deeply situated parts of the brain were much more changed, especially in the region of the large grey basal ganglia. Here the vessels were surrounded by a thick layer of lymphocytes, filling up the perivascular space and extending into the brain substance. Amongst the exuded lymphocytes there were a good number of red cells. There were also cells resembling granulation-tissue cells, hyaline with a vesicular nucleus; large cells (thirty to fifty μ in diameter) with protoplasm stained reddish with eosine and having one to three nuclei; a few phagocytes and an occasional plasma cell.

The lumina of the vessels were often narrowed. Close to the vessels were various sized haemorrhages without any lesions of the vessel wall.

Very often, external to the layer of white cells which surrounded a vessel, was a second layer of red blood cells infiltrating the brain substance in the neighbourhood. In some places the brain was altogether destroyed through haemorrhage, but no blood pigment was seen, and red softening was marked. Around the infiltrated vessels was usually a proliferation of glia cells. The endothelium of the vessels was proliferated and the vessels contained many white blood corpuscles, sometimes in so large numbers that they seemed to fill the whole vessel like a thrombus. The infiltration was in general equally pronounced both around artery and vein, but sometimes the vein was more changed than the artery.

Here, and in other sections, where the vessel was seen in tangential section, red corpuscles were present in the media and adventitia. The ependyma of the lateral ventricle was proliferated, forming a dense fibrous layer.

The epithelial cells of the choroidal plexus approached the columnar in type and often had gaps between them.

Similar changes to those in the cerebrum were found in the pons, medulla, and spinal cord.

The pons on section appeared much congested, the vessels were irregularly sheathed with a layer of cells of the same nature as those above described; the intervals between them being occupied by coagulated fluid. Throughout the section of the pons were seen various-sized haemorrhages; the endothelium of the vessels was thickened, and there were a fair number of white blood cells in the lumen. Similar changes but

more pronounced were found in the medulla—small-celled infiltration around the vessels, with a dilated perivascular lymph space, filled with transudate sometimes containing lymphocytes. The vessels in the white matter were not so much altered as those in the grey. Haemorrhages were also present in far greater numbers in the grey matter though no pigment was seen.

The pia and arachnoid of the spinal cord was the seat of a well-marked small-celled infiltration, the meshes in the connective tissue of the arachnoid being often altogether obliterated by it. It is worthy of note that in this region the walls of the larger vessels were very often normal, whilst the small ones showed marked changes.

The spinal dura mater presented the same change as the cerebral. The congested vessels ran between the connective tissue, in places surrounded by a few lymphocytes, and here and there even small collections of lymphocytes.

The inflammation continued along the vessels into the fissures and white substance of the cord. Both fissures were often indicated by a dark blue band, this appearance being produced by the accumulation of exudation cells alongside the vessels running into the fissures. The changes in the grey matter of the cord were also much more marked than elsewhere. The periphery of the cord was very oedematous, and showed proliferation of the neuroglia. The grey matter was often intersected by a network of congested capillaries, which were surrounded by a thick sheath of cells (consisting of elements similar to those of the brain vessels). The congestion of the vessels was so great that the vascular supply of the cord was displayed in the sections almost like a diagram. The perivascular changes were irregularly distributed, now one, now another vessel showing them. Relatively, few white cells were found in their lumen, and but slight signs of endarteritis. The epithelium of the central canal was proliferated, as also the neuroglia adjacent to it.

The most marked changes were found in sections of the spinal cord taken from the level of the sixth cervical down to the third dorsal segment. In the seventh cervical region, the artery of the left posterior horn was surrounded by a layer of lymphocytes, four to six deep, with a layer of red blood corpuscles of the same width, external to it again. At the base of the horn a haemorrhage destroyed its substance in half its width. In the grey commissure also an abnormal patch was seen, consisting of two large arteries with veins and a number of capillaries, all sheathed in infiltrated connective tissue.

Fig. 8 represents a section of the cord taken two segments lower down. The artery of the posterior fissure is seen enclosed in small-celled infiltration in the inner third of its course. There is also a haemorrhage on both sides of it extending into the white substance of the cord, and destroying the adjacent medullated fibres, so that there are only a few bundles of fibres left, which appear in isolated groups in the haemorrhagic region. Other smaller haemorrhages are also seen in the posterior horns and in the grey commissure.

All these haemorrhages were of quite recent origin, there being no trace of blood pigment. Below the third dorsal segment there were only some extravasated corpuscles around the infiltrated vessels. The other parts of the spinal cord all showed changes similar to those of the cervical part to a varying extent. Filaria embryos were present here and there in the vessels of brain and spinal cord.

The nerve fibres often showed alterations. The cerebral convolutions presented a marked degeneration of the supra- and intraradiar fibres. Usually there was slight degeneration of the fibres all over the brain, most marked around the larger foci of infiltrated vessels, the degeneration being both of older and more recent date. The fibres were often swollen and disintegrated. The pons and medulla showed degeneration of the medullated fibres irregularly distributed over the whole section, well marked around the infiltrated vessels, but most pronounced in the region of the haemorrhages, where MARCHI's method showed an extensive recent degeneration. The spinal cord presented the same condition, but certainly more degeneration in the posterior than in the lateral columns, and less still in the anterior columns.

The large nerve cells of the brain and spinal cord which contained a fair amount of pigment, showed very marked changes, not confined to a certain group, but irregularly distributed in the whole central nervous system. The cells in the basal ganglia and of the anterior cornua of the cord were in an advanced stage of alteration. In the neighbourhood of normal nerve cells were others whose processes seemed to be broken away. They were spherical in shape with a dilated pericellular lymph space. They often showed chromatolysis, the NISSL bodies were nearly disintegrated, and a powdery mass occupied the whole body of the cell instead. Sometimes the chromatolysis was only peripheral, the NISSL bodies in the centre well preserved ; at other times, the change was only central, in the periphery the NISSL bodies being easily distinguishable, and the centre occupied by a homogenous mass. The nucleus was often normal, but at other times had an irregular contour, or was even not distinguishable at all. Here and there were nerve cells with vacuolated protoplasm. Some of the nerve cells were oedematous, others were shrunken, stained dark blue, and in a later stage represented by a number of disintegrated fragments ; they were pyknotic. At various levels in the cord, the number of the large anterior root cells seemed to be unequal on the two sides.

The nerve bundles coming off from the spinal cord also showed great dilatation of their vessels, sometimes forming more than half the bulk. Frequently there was slight degeneration of the fibres and the vessels were surrounded by a thin layer of lymphocytes. The spinal ganglia at the various levels showed changes analogous to those in the cord, viz., small haemorrhages between the ganglion cells, and slight round-celled infiltration of the vessels. Haemorrhages were also present in the surrounding fat.

In the peripheral nerves were sometimes signs of neuritis, small-celled infiltration in the endo- and perineurium, and slight degeneration of the fibres.

With the above-mentioned bacteria-staining methods, microbes were only found in a few sections of the central nervous system, although very many sections were examined. They consisted of a few long bacilli in short chains and groups, and a few large cocci. The specimen stained by GRAM's method showed no micro-organisms.

B.—THE SECOND CASE, TOMI ; DIED JUNE 17, 1904

Post-mortem three hours after death.

Length one hundred and fifty c.m. ; very emaciated ; slightly built ; skin dry ; desquamating ; no particular pathological changes ; Rigor mortis commencing in upper extremities. Skull dolicho-cephalic. Pupils unequal, the right one smaller. Left eye showed a corneal opacity one mm. in diameter. Right eye, corneo-scleral junction at its right lower quadrant, ill defined, with peri-corneal injection corresponding. Conjuctivae and sclera pale ; slightly jaundiced. Mucous membrane of lips and mouth very pale. Neck short, thin, its hollows well marked. Glands on both sides of the sterno-mastoid muscle visible as slight elevations. Thorax barrel-shaped ; deeply indrawn intercostal spaces. Inguinal glands much enlarged ; skin over them elevated. External genitals normal.

The under surface of scalp pale, here and there dark-blue, dilated veins visible. The circumference of skull fifty cm. ; skull cap thin with very numerous pacchionian depressions, especially along the coronal suture. Dura not tense, adherent to the bone ; inner surface smooth and glossy, no thickening nor haemorrhages ; all its sinuses filled with dark fluid blood. The amount of cerebro-spinal fluid was increased. The capillaries were injected ; the pia mater, especially along the edges and over the occipital lobe was opaque and thickened.

The base of the brain presented the same changes in the leptomeninges. Arteries not atheromatous. The gyri and sulci normal. The brain substance on cross section soft and oedematous. All over the cut surface numerous congested capillaries and a few small haemorrhages were visible.

The ventricles were somewhat dilated ; the ependyma smooth and firm. The spinal dura mater appeared normal. The spinal canal contained much yellow-coloured cerebro-spinal fluid in which were found numerous leucocytes and a few trypanosomes. The anterior and posterior spinal arteries with their corresponding veins could be followed as thick cords along the spinal cord and even traced to their last ramifications.

On the anterior surface of the cord, on the right side, was a spindle-shaped dark red-stained mass (seven mm. in breadth) extending from the sixth cervical to the third dorsal, sharply defined and resembling a blood clot (Fig. 4). The cauda equina was embedded in gelatinous tissue. On cross-section the central canal was dilated ; the structure of the cord was well preserved ; the vessels in the grey substance and

those entering the cord were much dilated. The congestion was less marked in the lumbar region. At places a greyish stripe of infiltration was visible at the sides of the vessels.

Tympanic and nasal cavities were normal.

Thyroid normal. Both tonsils were enlarged but not actively. The glands of the neck were enlarged (four cm. long, two cm. broad); on cross section they presented a partial haemorrhagic infiltration, being brownish-red in colour and speckled with grey points the size of a pin's head (Fig. 5). The connective tissue at the hilus was hyperplastic. The submaxillary lymphatic glands were also haemorrhagic. Lungs were free from adhesions and much congested. In the pleura of the right lung numerous haemorrhages were present. The peri-bronchial glands were enlarged, showing the same changes as the glands of the neck.

The pericardium contained six c.c. of yellowish, blood-stained serum. The heart was of normal size, pale, and flabby; the epicardial fat well defined from myocardium. The cavities contained much dark fluid blood. The bicuspid valve was a little thickened, the other valves and aorta were normal. The mucous membrane of oesophagus and trachea was pale.

The abdomen contained a few c.c. of clear fluid. The liver was congested but otherwise appeared normal. The capsule was not thickened. The gall bladder contained much dark greenish bile. The spleen was increased in size (18 × 11 × 4 cm.), of a dark-reddish purple colour, hyperaemic, of solid consistence; capsule thickened.

The malpighian bodies appeared hyperplastic on section, the medullary substance showed small white striae, but was otherwise normal. The bladder contained cloudy urine, its mucous membrane pale. External genitals normal. Stomach and intestine showed a little congestion. Slight enlargement of lymph follicles and PEYER's patches could be made out; no cicatrices nor ulceration. The mesenteric glands were of a somewhat haemorrhagic appearance. The inguinal glands were the most enlarged (5 × 3 cm.), and showed the same changes as the cervical glands. Pancreas and supra-renals normal.

The bone marrow of the femur was reddish, with irregular greyish patches disseminated in it.

HISTOLOGY

On microscopical examination the organs showed lesions very similar to those of the previous case.

The *Epicardium* showed a varying amount of small-celled infiltration, most marked in the neighbourhood of the larger vessels and continuing along them into the myocardium. The infiltration consisted of leucocytes with a fair number of eosinophile cells and numerous red blood corpuscles. The endocardium also showed small-celled infiltration with similar constituents as in the epicardium. The striation of the muscle

fibres was frequently indistinct, and showed accumulations of brown pigment around their nuclei.

The *Lung* presented a remarkable congestion. The vessels of the alveolar septa were cork-screw shaped, projected into the alveoli and contained plenty of large mononuclear leucocytes. In some places were signs of commencing pneumonia. The alveoli were filled with a varying amount of exudation containing eosinophiles, and a large number of red blood corpuscles. There was striking hyperplasia of the lymphoid tissue around the bronchi. In the inflamed parts of the lung were a varying number of diplococci (stained by GRAM's method) and a few bacilli.

The *Liver* showed a little hyperplasia of the connective tissue. The cell rows were attenuated ; of the nuclei some were only faintly stained, others unstained ; the protoplasm being often merely an accumulation of various-sized fat droplets. No pigment was visible. Here and there were accumulations of red corpuscles between the liver cells.

The *Kidney* showed hyperaemia to such an extent that in some small places there was no epithelium to be seen, but only a delicate connective tissue with its meshes filled with red blood corpuscles. The glomeruli were often half-filled by dilated capillaries. Around the vessels were haemorrhages. Here and there parenchymatous degeneration was pronounced, the tissue being transformed into a homogeneous mass, with occasional tubules and a few epithelial cells left, and the whole traversed by numerous congested vessels containing many leucocytes.

The *Spleen* showed hypertrophy and hyperplasia of its follicles. The malpighian bodies were sharply defined and showed a light centre of large, irregularly-shaped cells with vesicular nuclei, other cells with much protoplasm and numerous (six to eight) irregularly situated, segmented nuclei, and a few mono- and polynuclear eosinophiles—all these cells being held in a fine meshwork of connective tissue. Around this centre is a corona of very densely-packed lymphocytes, while the external part is made up of the same interspersed with red blood corpuscles and polymorphonuclears. The congestion is very noteworthy, especially in the peripheral parts, where often nothing is visible beyond very thin sinus walls, surrounded by innumerable red cells. The sinuses contain many leucocytes, with a few phagocytes among them. The difference in staining reaction of the red cells is striking. In the VAN GIESON stained specimen, some of the corpuscles took the picric acid very intensely, others being only faintly or not at all stained. Sometimes they were clumped together and showed small iron-containing pigment granules ; these being found all over the spleen, and both free and intracellular, as for example, in the endothelial cells of the sinuses. The arteries show proliferation of their endothelium, and contain a fair number of leucocytes and a few megaloblasts. The spleen tissue contained phagocytes (with included red blood corpuscles and nuclei of lymphocytes), plasma cells, many eosinophiles, and giant cells of mononuclear type.

The *lymph glands* show generally the same changes as in the first case. The majority of the glands are affected, and all stages can be followed out from normal gland structure to that of haemo-lymph glands.

The following is a description of a typical haemo-lymph gland taken from one of the enlarged glands of the neck :—

There is a marked hyperplasia of the connective tissue, the capsule is thin and has enlarged vessels running in it. Underneath the capsule is the peripheral sinus, which does not extend round the whole gland, since at places the lymphoid tissue reaches the capsule. In the sinus are many red blood corpuscles, a few leucocytes and phagocytes and coagulated fluid ; from this sinus branches run towards the centre. The germ centres are in greater number than normally and are enlarged. They show nothing abnormal in their constituents except an infiltration of blood cells. The vessels often have an accumulation of red cells around them. The arteries inside, and to a less extent outside the glands show endarteritis. In some of the glands, chiefly in the periphery, are ill-defined strands of densely packed lymph cells. Similar patches, but smaller, are found in the central parts of the gland. A good number of phagocytes, a few giant cells, and a varying number of eosinophiles are found in the glands.

The vessels in the adipose tissue about the glands often have small new growths of lymphoid tissue around their walls.

Those lymph glands which appear slightly changed to the naked eye are found microscopically to be highly congested. Thick vessels enter at the hilus and divide into large branches running towards the periphery. Numerous free red blood cells are present among the lymphocytes throughout the glands and also there is a slight quantity of blood pigment giving an iron reaction.

The *long-bone marrow* in some places has the microscopical appearances of red marrow. It is very rich in cells, with but little fat. The vessels are very congested and contain a good number of leucocytes and a few nucleated red cells. The cellular elements present no abnormality, consisting of giant cells, eosinophiles, and a few phagocytes. Blood pigment is present in small quantity.

Eyes show microscopically nothing abnormal except a little infiltration at the corneo-scleral junction and in the cornea. The pial sheath of the optic nerve contains more cellular elements than normal, and the vessels of the nerve show well-marked small-celled infiltration.

Suprarenals are very congested. Enlarged vessels run in the capsule and between the cells separating them.

The *aorta* occasionally shows a little small-celled infiltration around its *vasa vasorum*.

The *central nervous system* presents much the same changes as in the case of 'Kitambo,' but as a rule in a less marked degree.

The leptomeninges are inflamed in varying degrees, the cellular infiltration being chiefly around the vessels, where it consists of a thick layer of lymphocytes; some of the vessels however show no such infiltration around them. This infiltration is found along the vessels running in the sulci, and it is made up of lymphocytes, a few large mononuclears, phagocytes, with peripherally situated nuclei and all sorts of inclusions, a varying number of red blood corpuscles, and cells having the appearance of granulation tissue cells. The infiltration to a certain extent accompanies the vessels into the cortex itself, being usually more marked in its deeper portions, especially in the large grey ganglia. The perivascular space is in places dilated and contains transudate.

In this case also the changes were most marked in the basal ganglia, where a very extensive layer of infiltration was found around nearly all the vessels. The vessels contain a good number of white blood cells, and in the larger arteries sometimes the whole wall contains blood corpuscles interpolated among its own normal constituents.

The perivascular infiltration consists mainly of lymphocytes, a few large mononuclears, granulation tissue cells, and a few plasma cells. External to the layer of cells often occurs a large space filled with transuded fluid. Usually the endothelium of the larger vessels is normal, while that of the smaller ones is proliferated, the intima appearing as little projections into the lumen of the vessel.

The infiltration extends to the brain tissue in the neighbourhood of the vessels, destroying it. The neuroglia is markedly proliferated in places. There are only a very few capillary haemorrhages.

In the *choroid plexus* there are many gaps between the cells and a slight inflammation of the connective tissue. The cells themselves, however, show very little change. The *pons and medulla* show the same perivascular changes as in the cerebrum, with occasional capillary haemorrhages. The meningitis is most pronounced over the cerebellum.

The spinal cord and its membranes show the same changes as the brain. The pia and arachnoid are more or less infiltrated with round cells, the infiltration following the vessels into the substance of the cord. The pia shows a haemorrhage into its substance at the level of the first dorsal. The connective tissue in the pia is loose owing to extravasated red blood corpuscles.

The peripheral part of the cord is oedematous, and its neuroglia proliferated. Around the vessels of the cord the small-celled infiltration is much more marked in those found in the grey substance than in the white; groups of six to ten vessels sheathed in a dense layer of lymphocytes are often observed. The neuroglia is a little proliferated. Various sized small haemorrhages are very often found in the grey matter, especially in the posterior cornua, often involving them to more than one-third their breadth; no blood pigment present. The nerve roots are also the seat of small haemorrhages. The central canal is dilated, and its epithelial cells

proliferated. The vessels are generally very much congested, and show a thickening of the intima. The perivascular infiltration often seems to compress the vessels.

The *nerve fibres* show little degeneration, and that mostly around the infiltrated vessels.

The large *nerve cells* of the brain and cord are greatly altered. The changes occur in nearly all groups. Somewhat irregularly distributed side by side with normal cells are others which do not contain any NISSL bodies, and appear disintegrated into a dust-like mass. Sometimes only a peripheral zone of NISSL bodies remains, at other times they are only present in the centre so that all stages of chromatolysis are seen. Often the nucleus is well preserved, at other times it is not distinguishable at all. Irregular, fragmented cells are met with, their processes seeming to have been broken off. Their protoplasm, especially of the cells of the anterior cornua, is often quite filled with small vacuoles.

The *spinal ganglia* sometimes show the same small-celled infiltration around the vessels as in the nervous centre. Small haemorrhages are found here and there.

Many of the *peripheral nerves* were also examined. Some, such as the sciatic and median, appeared normal; others, for instance, some branches of the lumbar plexus, vagus, and others, showed a more or less pronounced small-celled infiltration of the peri- and endoneurium.

Very many sections of all the organs were examined for bacteria with the above-mentioned methods, and, although very carefully searched, yet only a small number of large bacilli and large cocci which did not stain by GRAM's method were seen.

In the examination for trypanosomes in only a few sections of the brain, for example in the vessels of the choroid plexus, were a small number of trypanosomes found.

C.—THE THIRD CASE, BOYO

A black boy, fourteen years of age, died the 25th of May, at six p.m. The examination was not made until the 26th, at five p.m., twenty-three hours after death.

Body was very emaciated with sunken orbits and temples and deeply indrawn intercostal spaces. On the soles of both feet were numerous ulcers caused by chiggers. The limbs were much wasted. Rigor mortis was marked. Over the sacrum was a superficial bed-sore, ten c.m. in diameter. The skull was dolichocephalic, measuring fifty c.m. at circumference. The *dura mater* very adherent to the skull, which was of normal thickness, having deep sulci for the vessels and numerous small depressions for the pacchionian bodies. The dura appeared normal, and its sinuses contained a fair amount of clotted blood. The surface of the brain was of normal configuration, the veins of the pia very congested and filled with dark clotted blood, and showed opaque white bands along the vessels and thickenings in places.

The base of the brain was normal; the brain substance firm, and the vessels of the grey matter slightly congested; ventricular system dilated, its ependyma glossy, smooth and firm, showing congested vessels. The cerebellum presented the same congestion of its leptomeninges (Fig. 2), but appeared otherwise normal; pons and medulla macroscopically normal. The cord showed very congested vessels, the anterior and posterior spinal arteries appearing as thick coiling threads (Fig. 3). On cross section, hardly any change was seen except dilation of the central canal. The tympanic cavity was normal. Diaphragm on both sides reached the fourth rib.

Thyroid was a little enlarged, but appeared normal on section. The mucous membrane of the tracheae was swollen, congested, and of a dark red colour. Nearly the whole of the pharyngeal mucous membrane as far down as two cm. above the glottis was ulcerated, and covered with a stinking greenish-grey coating. The mucous membrane of the nasal cavities showed the same condition, and were filled by a greenish cheesy mass. The ulceration extended along the soft palate to the middle of the roof of the mouth. The uvula was oedematous and swollen. The mucous membranes of the frontal, ethmoidal sinuses and of the antrum were normal.

The *glands of the neck*, especially at the posterior border of sterno-mastoid, were enlarged (some three cm. in diameter), quite free, and on cross section presented a greyish meshwork filled with reddish-coloured tissue. Some of the glands, however, appeared normal.

Both *lungs* were free and not adherent. The visceral pleura was thickened and showed a few haemorrhages. The right lung was generally congested, with here and there patches of consolidation the size of a hazelnut. The mucous membrane of the main bronchus was livid, while the smaller bronchi contained pus. The left lung showed less consolidation than the right, and its main bronchus was only slightly affected.

The *pericardium* contained a few c.c. of yellowish-coloured serum. The heart muscle was pale and flabby. Endocardium, valves, and intima of the aorta normal. The peribronchial glands were slightly enlarged and anthracosed.

The abdominal cavity contained about two hundred c.c. of cloudy fluid. Liver was of normal size, pale and friable, the lobular arrangement indistinct. The gall-bladder full of dark brownish gall.

The *spleen* was enlarged (16 × 11 × 4 cm.), its capsule thickened, its tissue dark red in colour, very soft and easily torn. The malpighian bodies distinct and prominent and of a greyish colour. The urogenital system appeared normal. The mucous membrane of the stomach and intestines was pale; the latter contained a few examples of *Trichocephalus dispar* of both sexes.

The *mesenteric glands* were slightly enlarged, some being soft and haemorrhagic and others quite normal. Glands of the groin also enlarged (two-and-a-half cm.

long and one-and-a-half broad), reddish in colour on section. Pancreas normal. Suprarenals congested. The bone marrow of the femur was yellowish in colour, and showed several reddish islets.

Histology

The microscopical examination of the *heart* showed an inflammation of the epicardium of some standing. The myocardium showed patches of infiltration around its vessels, which were more numerous in the superficial parts, the muscle fibres being partially destroyed in their neighbourhood. The infiltration consisted of lymphocytes and numerous cells with abundant protoplasm and small round nuclei. The muscle fibres in places were fragmented. Haemorrhages occurred all through the muscle.

The *lungs* presented the appearance of catarrhal pneumonia; the alveoli were filled with blood cells and exudation, the vessels in the consolidated regions congested, and contained many white cells. The mucous membrane of the right bronchus was desquamated, and in the lumen was a purulent exudate. The lung structure had disappeared in some places, being replaced by exudation containing necrotic particles. The left lung showed similar changes, but to a less extent. Its bronchi contained a fair number of eosinophile cells. The lung tissue and the bronchi were filled with numerous bacteria and cocci, of which many stained by Gram's method.

The sections of the *liver* showed congestion, which was more marked in the periphery than in the centre. The connective tissue around the vessels was increased and infiltrated with lymphocytes. The liver cells were atrophied and filled with fat droplets and their nuclei had disappeared.

The section of the *kidneys* showed cloudy swelling. In its congested vessels are many white corpuscles.

The *spleen* showed considerable alterations of its structure. There was a striking congestion of the whole organ. In the section stained with polychrome methylene blue, stained areas of lighter appearance with a few nuclei in them and darker stained ones consisting of lymphocytes occurred. The light stained areas were the congested parts. The endothelium of the arteries was proliferated. The follicles were hyperplastic, and from them processes extended into the pulp tissue around. The cellular elements were as follows:—Normal spleen cells, numerous phagocytes, giant cells with a polymorphus nuclei and large protoplasmic bodies, and, in addition, a good number of large cells with peripherally placed nucleus and very vacuolic protoplasm. Iron containing pigment, both intracellular and free, was found all through the organ.

The *lymphatic glands* presented the same microscopical appearances as in the other cases, but the changes were not so extensive. Besides congestion, some of the glands showed the same sinus formation as has been described above. These sinuses were filled with red blood corpuscles and large phagocytic cells. Glands with this sinus

formation were found in all the groups. Very often the lymphoid tissue was not confined by the capsule, but was also found external to it, and lymphocytes were also interspersed in the adipose tissue. The germ centres in some of the glands were normal, but in others they were broadened out with numerous gaps in their continuity, whilst in others still they were not recognizable at all. The lymphoid tissue was very often interspersed with a large number of red cells, so that in the specimen stained with methylene blue lighter areas of unstained red cells and darker ones of lymphoid tissue were met with. The vessels often had accumulations of red cells around them, and in their lumen many white corpuscles.

Among the various cells found inside the sinus and outside it were very numerous phagocytic cells with red blood corpuscles, which in the specimen stained with modified LAVERAN'S method, were deeply stained orange ; other cells were mono- and poly-nuclear leucocytes with eosinophile granules ; cells of the size of large mononuclear leucocytes having small nuclei and basophile granules ; giant cells in limited numbers ; occasional cells such as are found in bone marrow, of an irregular oblong shape, staining a peculiar dark blue colour with haemotoxylin and containing one or more vacuoles, the protoplasm presenting a meshwork with one or two darker-stained spots in it ; and a few plasma cells. Blood pigment, which gives the Prussian blue reaction, was present in nearly all the glands.

The sections of the *bone marrow* of the femur showed the ordinary constituents of marrow ; nucleaed red cells were present in small number. The vessels were congested, and around them were very often accumulations of red blood corpuscles. Some parts presented typical gelatinous degeneration. In one place in the medullary substance of the left suprarenal was an infiltration of lymphocytes. The testes and epididymis were normal.

Brain and spinal cord showed the same changes as in the previous case, but to a far less extent. The pia and arachnoid over the cerebellum and over the convexity, more especially, of the cerebrum, presented a small-celled infiltration. The vessels were highly congested. The small-celled infiltration accompanied the vessels into the cerebral cortex. Many of the vessels of the cortex, however, were free from this perivascular infiltration, and only showed enlargement of the perivascular lymph spaces. The vessels of the grey basal ganglia were the most affected. The perivascular lymph spaces were occupied by a cellular accumulation consisting of lymphocytes, red blood cells, and a few plasma cells. The same changes were observed around the vessels of pons and medulla, while in the cord they were very little marked ; here the vessels have often a much-enlarged perivascular lymph space with a few lymphocytes in it. The cord showed a gliosis of its central canal. The nerve fibres presented very little degeneration, while the nerve cells, however, showed different stages of chromatolysis and pyknosis, more pronounced in the brain, however, than in the cord. The bacteriological staining methods revealed a varying number of large

bacilli and large cocci, which did not stain by GRAM's method, in all the organs. They are to be regarded as the result of *post-mortem* contamination.

Trypanosomes were not seen either in the organs or in the central nervous system.

D.—THE FOURTH CASE, DISASI

Well-built, with a fair amount of adipose tissue. Externally, nothing of note. Brain and coverings normal. Right tympanic cavity filled with pus. The whole of right lung consolidated. The lower part of the left upper lobe with the upper part of the lower lobe were congested, and showed small haemorrhages in the pleura.

Liver somewhat enlarged and congested.

Kidneys normal.

Spleen enlarged. Capsule thickened. On cross section the pulp was found soft and showed no particular changes.

All the *lymphatic glands* were enlarged, some being dark-red with pin-head sized grey patches here and there.

The mucous membrane of stomach and intestines pale ; jejunum contained a few ankylostomes.

Microscopical Examinations.—Sections of the *lungs* showed typical fibrinous pneumonia and very numerous diplococci, staining by GRAM.

The *spleen* was very congested.

The *lymph glands* presented the same conditions as in the sleeping sickness cases, only they contained far more free blood corpuscles among the lymphoid tissues. The examination of the *brain* and *cord* did not show any of those changes which were found in the sleeping sickness cases, although a large number of sections of different parts were examined. The perivascular lymph spaces were much dilated. There was neither haemorrhage nor small-celled infiltration in them, but they were often filled with transudation fluid containing one or two free lymphocytes. The large nerve cells only showed such changes as are caused by hyperthermia.

The cerebral vessels contained a large number of diplococci staining by GRAM, and here, as well as in the tissues of the brain itself as in that of the cord, were a great number of large bacilli in small clumps and short chains, not staining by GRAM.

II. EXAMINATION OF ORGANS OF ANIMALS INFECTED WITH *TRYPANOSOMA GAMBIENSE*

We examined the organs of a large number of animals such as monkeys, dogs (including puppies), rabbits, guinea-pigs, rats, and mice, who had succumbed to infection, with various strains of *T. gambiense.*

The brains of some of the monkeys in which the infection had run a longer course showed a marked congestion of the vessels, both in the meninges and in the brain itself. Some brains, especially those in which the infection had run a rapid course, were very anaemic. Those of the latter group showed little or no microscopical changes ; those of the former groups, however, presented marked changes affecting the vessels.

The section of one of the chimpanzees' brains showed extreme congestion, especially in the basal ganglia. Fig. 9 is a section from the optic thalamus of this brain. It shows a vessel with a large perivascular space distended with red blood corpuscles and among them a fair number of leucocytes. The endothelium is a little proliferated. The same changes were also observable in many of the neighbouring vessels. The meninges did not show any changes. The pons, medulla, and spinal cord were normal. A *Rhesus* monkey presented, in the region of the left central gyrus, a haemorrhagic cicatrix. Its surface was depressed below the level of the surrounding brain, and was of a yellowish colour. Microscopically it showed destruction of the brain tissue, with much pigmentation at the bottom of the softening.

Two of the infected baboons microscopically examined showed congestion of the vessels of the brain and spinal cord. Microscopically there were haemorrhages around the vessels of the grey matter of the brain, a good number of lymphocytes being present among the red cells. The grey matter of the spinal cord showed many localized haemorrhages. A few of the *Rhesus* monkeys, dying after infection of long duration, showed perivascular changes of varying extent in the brain and spinal cord. Those cases which showed perivascular changes also showed changes in their nerve cells similar to those in the human cases. The processes of the cells were often broken away. Sometimes no nucleus was to be found, and the protoplasm appeared vacuolic.

In the more chronic cases of trypanosomiasis in dogs, rabbits, and guinea-pigs, haemorrhages were present in the grey matter of brain and cord in a limited number. The vessels also often contained a large number of white blood corpuscles, and leucocytes were also found in the haemorrhages. The meninges showed nothing abnormal except a few small haemorrhages. Animals dying more rapidly from the infection did not show these changes in the central nervous system. The other

organs showed lesions which varied with the intensity and duration of the infection. The heart often showed all the signs of a haematogenous myo- and epi-carditis. The lungs were often highly congested ; the parietal and visceral pleura had often small haemorrhages. In every case there was great congestion of the liver vessels. They contained a large number of leucocytes. There was proliferation of their endothelium, and they were often surrounded by small-celled infiltration. The liver cells showed fatty infiltration, and, at a later stage, fatty degeneration. Far advanced parenchymatous degeneration was frequently found. The kidneys did not show anything of note, except great congestion of the vessels and smaller and larger haemorrhages between the tubules.

The *spleen*, however, showed great changes. In almost all animals it was more or less enlarged, sometimes up to thrice its normal size. It was generally of a dark purple-red colour, and had a tense capsule. In cases dying after an acute course of infection its pulp was very soft, and the follicles large and very prominent. Microscopically, the hyperaemia was striking, being more pronounced at the periphery than towards the centre. In some cases small necrotic areas were present.

The spleen cells generally took the stain deeply. The follicles were hyperplastic and irregularly defined, with processes running out from them. They may be described as follows :—a centre of lightly-stained cells having the appearance of granulation cells, around it a dense layer of lymphocytes with numerous red blood corpuscles interspersed. The vessels running in the trabeculae showed proliferation of their endothelium. There were a large number of phagocytes with included red cells and here and there were leucocytes with eosinophile granules. The red blood corpuscles accumulated in the spleen often showed all the signs of degeneration. Blood pigment, giving the iron reaction, was also present, both intracellular and free.

The spleen of those animals which had died after a more chronic infection was in the majority of cases much enlarged, dark, and of firm consistence. The malpighian bodies, however, were not so prominent, but rather seemed to be lessened in number and in size. On microscopic examination, the trabecular system showed much hyperplasia. The whole organ was very hyperaemic, many of the blood cells showing degeneration. The phagocytes and leucocytes with eosinophile granules were increased in number. Some cases had a large amount of blood pigment.

The *lymphatic glands* were usually enlarged, and their stroma hyperplastic. In nearly all the groups besides glands of normal aspect there were others reddish-brown or dark-brown in colour, which presented intersections of greyish bands corresponding to the hyperplastic connective tissue. On microscopical examination they showed sinus formation throughout the gland. The sinus contained many red and white blood corpuscles, phagocytes, which had included pigment, and the granular remains of red blood corpuscles. The lymphoid tissue often had many free blood corpuscles interspersed through it, along with a varying number of eosinophile cells, pigment, and

giant cells. In some glands the lymphoid tissue was reduced to smaller or broader bands, between which were lightly stained strips of finely reticulated connective tissue having in its meshes a varying number of blood cells and large hyaline cells packed with pigment and very numerous free pigment granules.

The long-bone marrow sometimes presented the appearance of red marrow. Microscopically it occasionally showed slight gelatinous degeneration.

In animals having large numbers of trypanosomes present in their peripheral blood at the time of death trypanosomes were easily detected in the sections of the organs (Fig. 10).

They were found both singly and in groups in the lumen of the vessels along with numerous leucocytes. Only in the spleen, however, were they found outside the vessels among the tissue cells. For staining the trypanosomes we used a modification of LAVERAN's method, which we found to give the best results for tissue imbedded either in paraffin or celloidin.

The section is stained in a mixture of

1 c.c. Borrel blue
4 c.c. Eosin (1 in 1,000 solution)
6 c.c. Distilled water.

Stain for half-an-hour or a little longer. The section will now be of a dark-blue colour. Wash for a short time in distilled water, and differentiate with orange tannin (Unna), wash again well in water till it appears purple-red in colour, then pass rapidly through absolute alcohol into xylol and mount in *neutral* Canada balsam. A very easy method of dehydrating is to use aniline oil. The sections after differentiation with orange tannin and washing with water are fixed upon the slide. The excess of water is removed by blotting paper, and pure clear aniline oil is added two or three times for a very short time. After removing the oil, the sections are passed through xylol and mounted in the balsam. Both methods by careful management give the same good results. The protoplasm of the trypanosome is stained orange, and the chromatin of a red-violet colour.

The flagellum is unstained. Thin sections are needed to get good results. Thick sections stain blue throughout.

GAMBIAN HORSE TRYPANOSOMIASIS

For studying the lesions caused by *T. dimorphon* in the organs of experimental animals, microscopical examinations were made of monkeys, dogs, rabbits, guinea-pigs, and rats.

Macroscopically, the leptomeninges of brain and spinal cord were very highly congested in all the more chronic cases, showing dilated capillaries in a meshwork of large dark veins. Cross sections showed the same congestion, especially in the grey matter, in which were also a varying number of haemorrhages. The cerebro-spinal

fluid was generally increased in amount. The lungs were normal, the heart flabby. There was generally effusion in the pericardium and pleural cavities. The liver was usually much congested, its vessels enlarged and filled with both dark fluid and clotted blood. The capsule was very often thickened.

The *spleen* in all the animals was very much enlarged, being sometimes four or five times its normal size. On section it often showed haemorrhages, the pulp being dark-red in colour. The follicles were usually hyperplastic and prominent.

The *kidneys* appeared normal.

The *lymph* glands were enlarged as a rule, and whilst few appeared normal, most of them were light or dark-brown in colour with greyish intersecting bands of hyperplastic connective tissue.

The *bone marrow* in most cases was dark-red in colour with a few grey islets in it.

Microscopical Examination

In some of the vessels of the grey matter of the brain and spinal cord, the perivascular spaces were filled with red blood corpuscles, pigment granules, and a few leucocytes. The tissue around the vessels was often destroyed.

Spleen. The most striking feature was the extreme congestion which, in the majority of the cases, was most marked in the periphery, where sometimes nothing could be seen but red blood corpuscles with a few spleen cells here and there amongst them. The follicles were enlarged, and consisted mostly of leucocytes. In sections from the periphery, the cells often seemed to be arranged in cords along fine bands of connective tissue. The space between the cords was packed with red blood cells and phagocytes. Nearly all sections showed large numbers of hyaline cells containing red corpuscles and pigment. The more chronic the case the more blood pigment was found in the spleen, so that in all old cases the whole of the organ was filled with large granules, of which a fair number were intracellular. In some sections eosinophile cells were present in large numbers. There were also more or less extensive haemorrhages, the large ones destroying the tissue right up to the capsule, thus accounting for the ruptured spleen sometimes found. The vessels showed great proliferation of their endothelium, so that in places they seemed to be obstructed.

The *lymphatic glands* presented marked changes. Very often the lymphoid tissue was reduced to small cords traversing the gland. Beneath the capsule was a small layer of lymphocytes. The spaces between the bands of lymphoid tissue network contained a few lymphocytes, hyaline cells with two or more nuclei, cells with a large quantity of included pigment, giving the iron reaction, and very many large free pigment granules (see Fig. 11).

It is remarkable that in some cases in which the spleen contained only traces of pigment the lymph glands were practically filled with it. The amount of pigmentation in the glands and spleen increases with the duration of infection.

The *kidneys* were normal in most cases, sometimes they showed parenchymatous degeneration.

The *liver* cells were often wasted, sometimes showing fatty degeneration, and had no nucleus or only a faintly stained one. Blood pigment was often to be found in the liver.

The other organs showed nothing abnormal, except congestion of vessels.

Surra, Nagana, Etc.

We examined microscopically the organs of rabbits, guinea-pigs, and rats infected with *T. brucei* and *T. evansi*, and the organs of young dogs infected with *T. equiperdum*.

In the organs of animals infected with *T. brucei* we found in the brain and spinal cord congestion and a few haemorrhages around the vessels, as for the other organs, we can only confirm Baldwin's findings. We could not find a periodical variation of the pigment of the spleen, but the amount of pigment increased with the duration of the infection. In acute cases there were very often haemorrhages in the spleen destroying its tissue, and often extending up to the capsule. The liver showed small necrotic areas in places.[1] The lesions in Surra and Mal de Caderas were of the same nature, but showed slight variations. The amount of pigment in the spleen in these two latter diseases was always very small. Trypanosomes in groups were found in nearly all vessels.

The organs of one pup infected with Dourine were normal, as were the brain and spinal cord. The spleen was not much enlarged, and was of normal microscopical appearance. The lymph glands were greatly enlarged, however, the enlargement being due entirely to the new formation of lymphoid tissue.

Summary

The pathological histological changes of the brain and spinal cord in cases of sleeping sickness were first described by Mott, Warrington, and the Portuguese Commission. Low and Mott describe the case of a European. Mott has recently, in a lecture on the cerebro-spinal fluid, recorded the changes found in some more recent cases.

The changes described by all the authors are the small-celled infiltrations around the vessels of brain and spinal cord, especially remarkable through the presence of plasma cells. Some of the authors, especially Mott, have described diplococci as being present in the vessels of the central nervous system. In our three cases of sleeping sickness a few cocci and bacilli, negative to Gram, were seen in the brain, cord, and organs, but they were so few in number that they could be considered as due

1. This observation is confirmed by Neporogny and Yakimoff. Modifications du foie dans les Trypanosomiases experimentales (Recueil des Travaux dédiés a M. Lukianoff a l'occasion de son jubilé scientifique. St. Petersbourg, 1904).

to *post-mortem* contamination. The cases died during the summer months; the earliest autopsies were performed four hours after death.

We may draw attention to the fact that large quantities of pure blood or cerebro-spinal fluid taken during life from these cases were inoculated intraperitoneally into monkeys, guinea-pigs, rats, and mice, and intravenously into rabbits without causing any septic infection. As much as twenty c.c. of pure blood has been inoculated, and cerebro-spinal fluid in the amount of ten c.c. Cultures were also made from the blood and cerebro-spinal fluid of two of the cases of sleeping sickness, and were negative, with the exception of one or two tube and flask cultures, which showed a growth of *Staphylococcus albus*.

Undoubtedly a large number of sleeping sickness cases do not die from the original infection, but rather from some secondary infection, such as pneumonia, septic meningitis, etc., caused either through septic processes starting in the oral or nasal cavities or due to the generally debilitated condition, a result of the trypanosome infection. Among the experimental animals a heavy mortality occurs when any epidemic occurs among them. Haemorrhagic lymph glands have been described by DUTTON, TODD, and CHRISTY in their series of sleeping sickness cases. The two first observers have also described such glands in their experimental animals infected with *T. gambiense* and *T. dimorphon* (Senegambia report).

In all the three cases of sleeping sickness, and in the case of trypanosomiasis, and in many of the experimental animals infected with *T. gambiense* and *T. dimorphon*, haemorrhagic lymph glands have been found. These glands have all the characters of the haemo-lymph glands, as described by ROBERTSON, CLARKSON, VINCENT and HARRISON, WARTHIN, DALTON, KELLY, and other observers. These authors describe them as normally present, but in small numbers, in human beings and animals, and always in the neighbourhood of large blood vessels. We find that these glands are present in large numbers, and all stages between normal and haemo-lymph glands are to be seen, especially in the human cases. Necrotic areas were present in the spleens of our human cases and some of the animals. The spleens of all were intensely congested. Blood pigment giving the iron reaction has been found in the spleen and glands of these cases. It would seem from all these facts that an extensive destruction of the blood corpuscles occur and, as the bone-marrow is always degenerated, that there is not a sufficient formation of new cells.

Animals infected with various strains of trypanosomes derived from sleeping sickness and trypanosome fever cases show similar changes in their nervous system and organs as those described in sleeping sickness cases; and these changes depend largely on the duration of the disease; and, moreover, similar changes are described above in animals infected with *T. dimorphon* (but only after the disease has continued for a long time). Further, SIVORI and LECLER describe in their paper, *La Surra Americane* (Mal de Caderas), haemorrhagic areas in the grey substance of

the posterior cornua and small-celled infiltration around a blood vessel in the lumbar region of a horse naturally infected with the malady (it had shown paraplegic symptoms). Neuritis has been described in animals infected with *T. equiperdum*.

From these facts one must conclude that the lesions in the brain, cord, and organs can be produced by the trypanosomes. We have only found these changes occurring in animals infected with the parasite for some time, and in whom there were evidences of the duration of the disease, such as anaemia, loss of weight, etc. It would, therefore, appear that only in the chronic infected animals do the lesions occur.

In the 'trypanosome fever' case we have not found the same appearances in the brain and spinal cord as in our sleeping sickness cases, probably because the disease was not long enough established to produce the changes around the vessels of the nervous centres.

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ADDENDUM (see p. 13)

Note on the effect produced on rats by the trypanosomata of Gambia fever and sleeping sickness, by H. G. PLIMMER, F.L.S., communicated by Dr. C. J. MARTIN, F.R.S. Received December 1, 1904. Read January 19, 1905.—*Proceedings of the Royal Society*, T. LXXIV, No. 504, February 24, 1905, pp. 388-390.

Synopsis of the Paper :—

Gambian Fever. Fourteen rats

Parasites appeared about four weeks after inoculation. Duration of disease, two months twelve days. Parasites present in large numbers towards end. Paralysis. Nervous symptoms, none ; before death, animal heavy and apathetic.

Sleeping Sickness. Three rats

Parasites never found in the blood. Duration, six to nine months. Paralysis—

Both hind legs May 12, death May 23
„ „ Aug. 2, „ Aug. 30
„ „ Aug. 28, „ Sept. 8.

Post-Mortem

Spleens not enlarged. No microscopic lesions. The blood was citrated and centrifugalized, and the organs were mashed and washed with normal salt solution and centrifugalized, but in no case were any trypanosomata found. Portions of the extracts of liver and spleen and spinal cord were injected into other rats, but up to the present these show no sign of illness. *In an addenaum, December* 14, 1904. Two of the three rats inoculated developed paraplegia. The blood examination of these animals fails to discover any trypanosomata. Apart from the paralysis the animals show no sign of ill-health. In the mashed spinal cord of each of the rats the characteristic trypanosomata were found in small numbers, but none were found in the brains which were examined in the same way.

Mr. Plimmer concludes

1. 'These experiments go to show that the two diseases—Gambia Fever and Sleeping Sickness—from which the organisms were obtained, are distinct ; the duration of the disease, the symptoms and the *post-mortem* appearances being quite different. It is evident that these two organisms are quite separate and distinct, as their different effects on similar animals indicate. The fact of the clinical observation that Gambia fever not infrequently appears to terminate with all the symptoms of sleeping sickness may quite possibly be explained by a double infection. For, in both rats and monkeys, the one trypanosoma does not interfere with the other, but the more active organisms—that of Gambia fever in the case of rats and monkeys—kills in about the same time, whether inoculated before, with or after that of sleeping sickness.'

2. 'There can be no question, from the above experiments, of the susceptibility of the rat to the trypanosoma of sleeping sickness.'

3. 'These experiments show that the inoculation of the trypanosoma of sleeping sickness into rats gives rise to no obvious symptoms for many months, nor are trypanosomata discoverable in the blood by microscopic examination. But after a period of from six to nine months paraplegia occurs, leading to the death of the animal ; and *post-mortem* the organisms are found only in the spinal cord. The organisms are thus in rats, as sometimes in man, entirely confined to the nervous system ; where, as in monkeys, they are, in my experience, always generalized at some period of the disease.'

In this laboratory more than seventeen rats have been used in the research. The following tables show the behaviour of the parasite in a *few* of the rats infected with the various strains. The third column gives the record of the parasite as proved by microscopical examination.

EXPLANATION OF SIGNS

+ + + Very numerous, fifty or more to a field.
+ + One or more to a field.
+ Fairly numerous.
– Scanty.
o Absent.
p. Periodicity, the parasites disappearing or diminishing to reappear or increase.

Example—+, p, –, o, read :—
'+,' the parasites were fairly numerous at first.
'p,' periodicity then occurred, the numbers diminishing or disappearing to reappear.
'–' the parasites became less numerous, and
'o' at death the blood was negative.
Unless 'o' is recorded, understand parasites seen at death.

From these figures it is evident that the results of the inoculations of our many different strains are absolutely opposed to those of PLIMMER. As noted before, the strains used by PLIMMER are two of those used by us and Professor LAVERAN. The record of the direct inoculations and sub-inoculations of blood from 'Kitambo,' and of blood and cerebro-spinal fluid from 'Tomi,' *show that no such a feature* as no trypanosomes appearing in the blood but in the spinal cord, and later on, paralysis occurring was observed.

Mr. PLIMMER claims that in man the organisms are sometimes entirely confined to the nervous system. Careful examination of the blood in such cases would reveal the parasites. As pointed out under Periodicity of the Parasite in the Natives (see p. 10), the daily examination of the blood for many days is necessary before such a decision can be arrived at.

Until Mr. PLIMMER can show that he has been able to obtain his original results on a large series of rats inoculated with the Uganda and other strains of Sleeping Sickness, the question may well be raised : 'Must such a dogmatic conclusion, as this result of only seventeen experiments, be accepted as to the differentiation of the parasite of "Uganda" Sleeping Sickness from that of "Gambian Fever"?' The figures of this laboratory are entirely opposed to such a conclusion.

Uganda Sleeping Sickne: 'Gambia Fever.'—Subinoculations

Incubation --days	Duration days	Recor bation—days	Duration—days	Record of parasites in the blood
20	210	njur strain		
14	140	- 7	106	-, p, +
14	127	- 3	22	+, p, + +
9	129	- 7	134	+, p, -, o
5	11	+ 6	70	+, p, + + +
13	61	+ 1	72	+ +, p, -, +
5	29	+ 1	35	+, p, -
4	33	+ 10	33	-, +
5	76	+ 6	72	+, p, + + +
4	42	Q' strain		
		8	365	-, p, o
		min' strain		
		18	366	-, p, o, +

GLAND PUNCTURE IN TRYPANOSOMIASIS

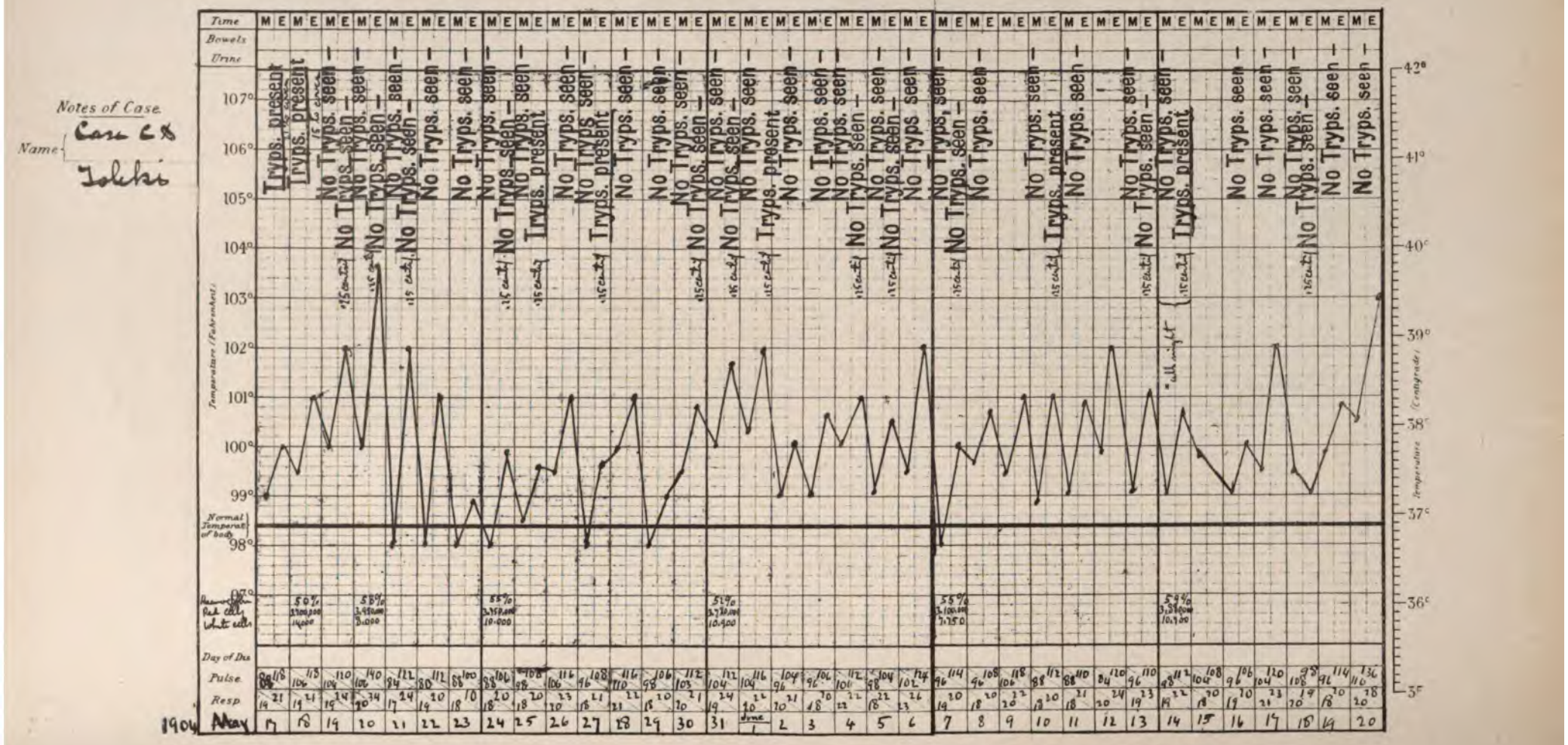

FIG. 1. Commencing at 9 a.m. on June 14 the blood was centrifuged at three hour intervals for a space of twenty-four hours. Parasites were found only at 9 and 12 a.m. Unless it is otherwise indicated the examinations noted were always made with fresh coverslip preparations of finger blood

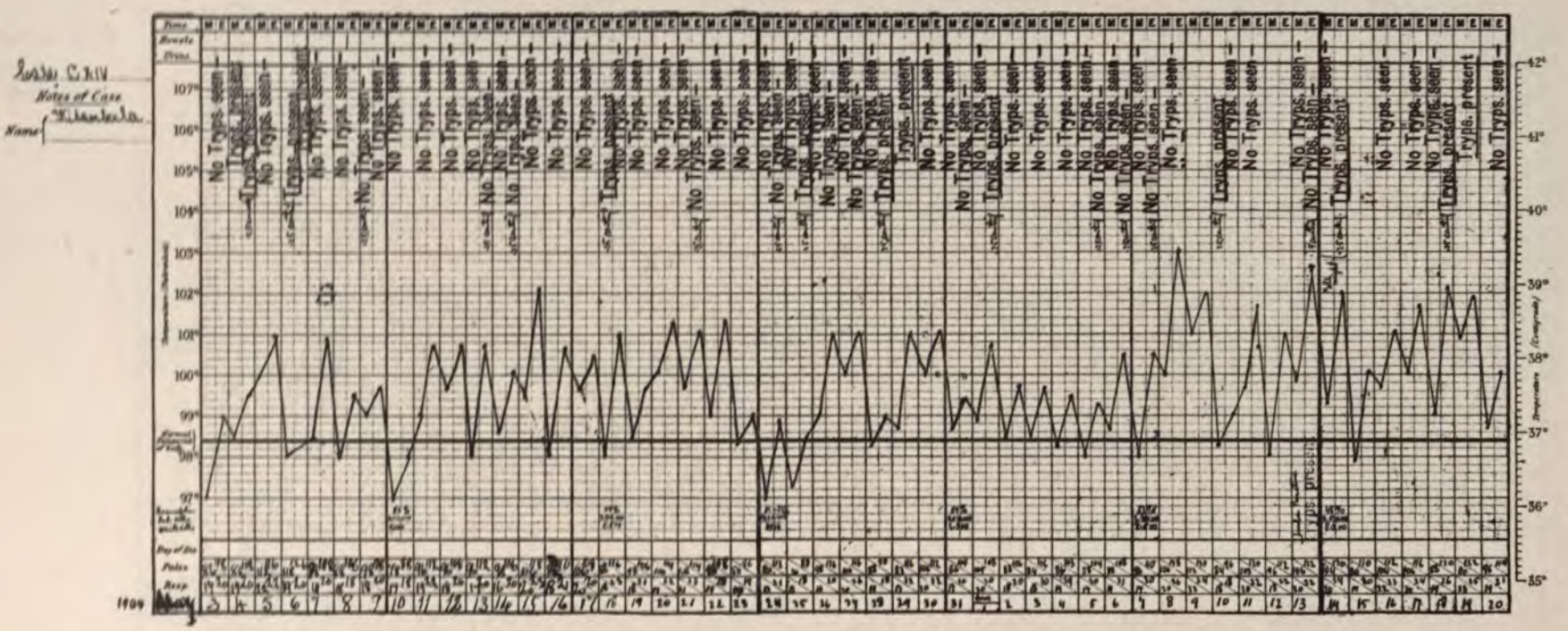

FIG. 2. Commencing at 9 a.m. on June 14 the blood was centrifuged at three hour intervals for a space of twenty-four hours. Parasites were found only at 9, 12, and 6 a.m. Unless it is otherwise indicated the examinations noted were always made with fresh coverslip preparations of finger blood

GLAND PUNCTURE IN TRYPANOSOMIASIS*

COMPARED WITH OTHER METHODS OF DEMONSTRATING THE PRESENCE OF THE PARASITE

FOURTH INTERIM REPORT

FROM THE EXPEDITION OF THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE TO THE CONGO, 1903

BY THE LATE

J. EVERETT DUTTON, M.B. VICT.

WALTER MYERS FELLOW, LIVERPOOL UNIVERSITY

AND

JOHN L. TODD, B.A., M.D. McGILL

THE great need of a good routine method of searching for the parasite in persons supposed to be infected with trypanosomes has long been apparent to us.

When the investigation of human trypanosomiasis was commenced it was thought, from the analogy of similar diseases in animals, that the microscopical examination of body fluids, particularly blood, and their experimental inoculation into laboratory animals would be the two methods which might be employed in the examination of suspected individuals.[1] Animal inoculations were soon found to be impracticable because of the uncertain and variable pathogenicity of *T. gambiense* to all ordinary laboratory animals,[1,2] and the examination of the blood alone was relied upon for diagnosis. Unfortunately, the parasites appeared only periodically in the peripheral circulation of even known cases, and often a prolonged search of many preparations was without result or only revealed a single parasite. Therefore repeated and tedious examinations of many preparations were necessary before a suspected case could, even tentatively, be said to be uninfected.

It was found that if infected blood were mixed with some diluent which prevented coagulation and centrifugalized, the parasites were deposited, along with the white corpuscles, between the red cells and serum.[3] Various methods of centrifugalizing large (5 c.cm.) or small (·5 c.cm.) quantities of blood were devised. During the past two years we have employed a simple method of centrifugalizing small quantities of blood which has given very satisfactory results. An inspection of the accompanying charts (Figs. 1 and 2) will suggest how frequently blood negative to coverslip examination has been positive when examined by this method.

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* Dr. Todd informs me that this paper was written at Nouvelle Anvers, in August, 1904. A copy arrived in Liverpool two months later, but was unfortunately mislaid, so that the publication of the article has been unfortunately delayed until now. (Signed) Ronald Ross, *Professor of Tropical Medicine, University of Liverpool.*

The apparatus necessary are an ordinary alcohol lamp, a high speed centrifuge, a few capillary pipettes, a diluting fluid—we used a 1·5 per cent. solution of sodium citrate in normal saline—and a special bulb-shaped tube capable of containing ·25 to ·5 c.cm. (Fig. 3). These tubes are easily drawn out in the flame of a bunsen from easily-fusible glass tubing having an internal diameter of a little more than five millimetres. They are afterwards cut with a file to a length which fits the haematocrit arm of the centrifuge.

It is very necessary for the successful use of these tubes that each step should be done quickly. The patient's finger must be well pricked so that the blood flows freely and in fair quantity. The longer arm of a perfectly clean tube—if it is greasy blood will not enter—is gently touched to the drop of blood and the tube allowed to half fill by capillarity—breathing through the tube just before using it will cause the blood to enter more easily. The tube is then quickly transferred to the diluting fluid and allowed to almost, never totally, fill itself. The filled tube is gently rotated so as to thoroughly mix its contents, and then, held so that there is an empty space at either end, the extreme tip of the longer arm is placed in the flame of an alcohol lamp and allowed to seal. If the mixture of blood and diluent becomes coagulated by heat, or if an air bubble forms in the tube before it is sealed, it will be found better to commence again than to attempt to remedy either defect. The best results are

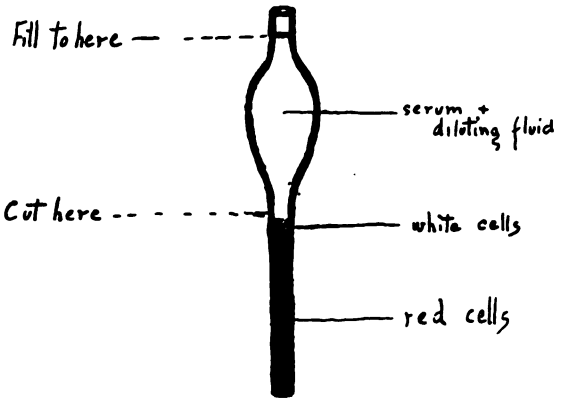

FIG. 3

obtained from tubes so prepared if they are first centrifugalized rather slowly and later at a much higher speed, for instance, for five minutes at eight to nine revolutions per minute of the handle of DELAND's centrifuge, and then for two minutes at sixty to seventy (about eight thousand revolutions per minute). At the end of this time a well-marked white ring will have formed above the red cells. The undesired, overlying, clear fluid can be quickly removed by gently scratching the tube at about three millimetres above the white ring with a file and then breaking off the bulb by a smart tap from the same tool. Any superfluous diluting fluid is first removed, and the

white cells carefully drawn up together with a small quantity of serum by means of a capillary pipette. If the operation is well done only sufficient material for one coverslip preparation is obtained.

Although such methods are exceedingly efficient, periods do occur during which the most careful examination fails to find trypanosomes in the blood of even advanced cases of sleeping sickness in whom parasites have previously been very numerous. (Fig. 1). Much less, then, can once or twice repeated negative examinations of blood from a merely-suspected person be accepted as proving non-infection.

The introduction of lumbar puncture in cases of sleeping sickness by CASTELLANI supplied a new and valuable diagnostic method, and not infrequently the deposit derived by centrifugalizing from fifteen to twenty c.cm. of spinal fluid showed parasites in individuals from whose blood they had been persistently absent.[4] In early cases of sleeping sickness, however, the conditions were frequently reversed, and trypanosomes were found in the blood, while examinations of the cerebro-spinal fluid remained negative.[5] Neither method, then, can be entirely relied upon. In addition, both spinal puncture and the centrifugalizing of blood require too much time and technique for either to ever become a commonly employed diagnostic method.

The examination of spleen pulp and serous fluids (hydrocele) gave, sometimes startling,[4] but no constantly positive, results.

Although it is scarcely worthy of being considered a diagnostic method, we mention a phenomenon[3] frequently observed in ordinary vaseline-sealed coverslip preparations of fresh blood from cases of trypanosomiasis. The red cells, instead of forming rouleaux as normally, run together into formless clumps and huge agglomerate masses. The condition is easily recognized macroscopically, and the sandy, granular appearance of the preparation is very characteristic. Although this condition is not always seen in infected bloods, and is sometimes observed in preparations from cases who have never been infected, still it has so constantly been associated with the presence of trypanosomes that bloods which 'agglutinate' in this manner are looked upon with the greatest suspicion. More than once has the 'agglutination' encouraged the continuance of an ultimately successful search for parasites. Only once[4] have we had the opportunity of observing a patient (European) from whose blood trypanosomes, once present, have finally disappeared. In this instance autoagglutination of the red cells disappeared with the parasites.

One of the great interests of the note[5] from Capt. GREIG and Lieut. GRAY, recently communicated by BRUCE, was that it suggested a new* and possibly more perfect method of demonstrating the parasite in early cases of trypanosomiasis.

By a comparison of the results obtained from the simultaneous examination of blood, gland juice, and cerebro-spinal fluid from a series of fourteen suspected, though very early, cases of 'sleeping sickness,' we satisfied ourselves that, as a rule, trypanosomes

* Kanthack, Durham, and Blandford[3] had already noted that parasites might occasionally be present in the glands, though absent from the blood, of animals infected with *T. brucei*.

were present in the few drops of fluid drawn by a hypodermic syringe from the enlarged glands of infected persons ; that parasites could be frequently demonstrated by this method where careful centrifugalizing of peripheral blood had failed ; and that the parasites were often seen in as great numbers in the preparations of gland fluid as in the preparations of the sediment obtained by centrifugalizing many (twenty) cubic centimetres of cerebro-spinal fluid.

These results were most encouraging, and it was resolved to test the method further by using it in the examination of apparently healthy persons. Twenty-two natives, coming from districts where the disease occurred, but all absolutely unsuspected of being cases of 'sleeping sickness,' were chosen because of their more or less enlarged glands. These were punctured and trypanosomes were found in the fluid aspirated in eight instances. A simultaneous examination of fresh coverslip preparations only detected two of these cases, and by centrifugalizing the blood only one additional case was revealed. Lumbar puncture was done on two of the cases found to be infected by gland puncture and the spinal fluid shown to be in every way normal. Although the largest cervical glands have usually, for convenience sake, been those punctured, parasites have been found, with equal facility, in glands no larger than peas and in glands from other groups.

Only twice during the examination of a series of thirty cases of trypanosomiasis did a single examination of gland fluid fail to demonstrate the parasite which other methods of examination showed to be present.

On seven occasions (in twenty-six cases) it was successful when centrifugalizing the blood had failed, and it was thrice positive (sixteen cases) where examinations of the cerbro-spinal fluid had given negative results.

Although a general glandular enlargement is very common among African negroes, it seems possible that, at all events in infective areas, persons with much enlarged lymphatic glands must, other causes being absent, be regarded as possible cases of trypanosomiasis. For example, fresh coverslip preparations of blood from a squad of twenty-six healthy soldiers were examined. Two men were infected. Both had been previously chosen, with three others, for gland puncture because of their enlarged cervical glands. Unfortunately, further examination of these cases was not permitted.

A small herd of cattle was established near Coquilhatville in 1902 by the Congo Government. The cattle suffered a good deal from an unrecognized chronic wasting disease, ending in death. The veterinary surgeon (M. BERTOLOTTI) in charge had made an excellent report to the Governor upon the clinical aspects of the disease, and there seemed to be some analogy between the symptoms which he described and those observed in Gambian horses and cattle infected naturally, or by inoculation, with *T. dimorphon*.[1]

When we arrived in Coquilhatville only one cow out of a herd of forty odd

head showed obvious signs of disease. The first examination of its blood was negative. Ten days later the blood was again centrifugalized, and trypanosomes were found. The fluid from a superficial neck gland was examined at the same time, and parasites, in fair numbers, were seen to be present. Three days later the animal was killed and an autopsy performed. Parasites, though once more absent to ordinary coverslip preparations of blood, were again found by centrifugalizing. They were also seen in the juice of a hyperaemic mesenteric gland, which was examined almost immediately after the animals death, in about the same numbers as at the previous examination of cervical gland fluid. After this positive result it was extraordinary that many preparations, made one or two hours after death from glands taken from various parts of the body, should have all been negative, while preparations from blood, pleural, pericardial, and peritoneal fluids all showed active parasites.

This observation has a most interesting parallel in the results of our autopsies on cases of sleeping sickness.[4] Only once,[4] and that in an autopsy done within an hour after death, have trypanosomes been found in gland fluid taken *post-mortem* from cases of human trypanosomiasis, but living parasites have very frequently been seen in the various serous fluids.

The bloods and gland fluids of several other animals from this herd were examined with negative result.*

We conclude that :—

(1) The examination of glandular fluid, though not infallible, is a very efficient means of detecting the presence of trypanosomes,

(2) Because of its simplicity gland puncture will be found a very useful routine diagnostic method.

* The neck glands of two cows, infected with trypanosomiasis, since examined at Nouvelle Anvers, were found to contain parasites.

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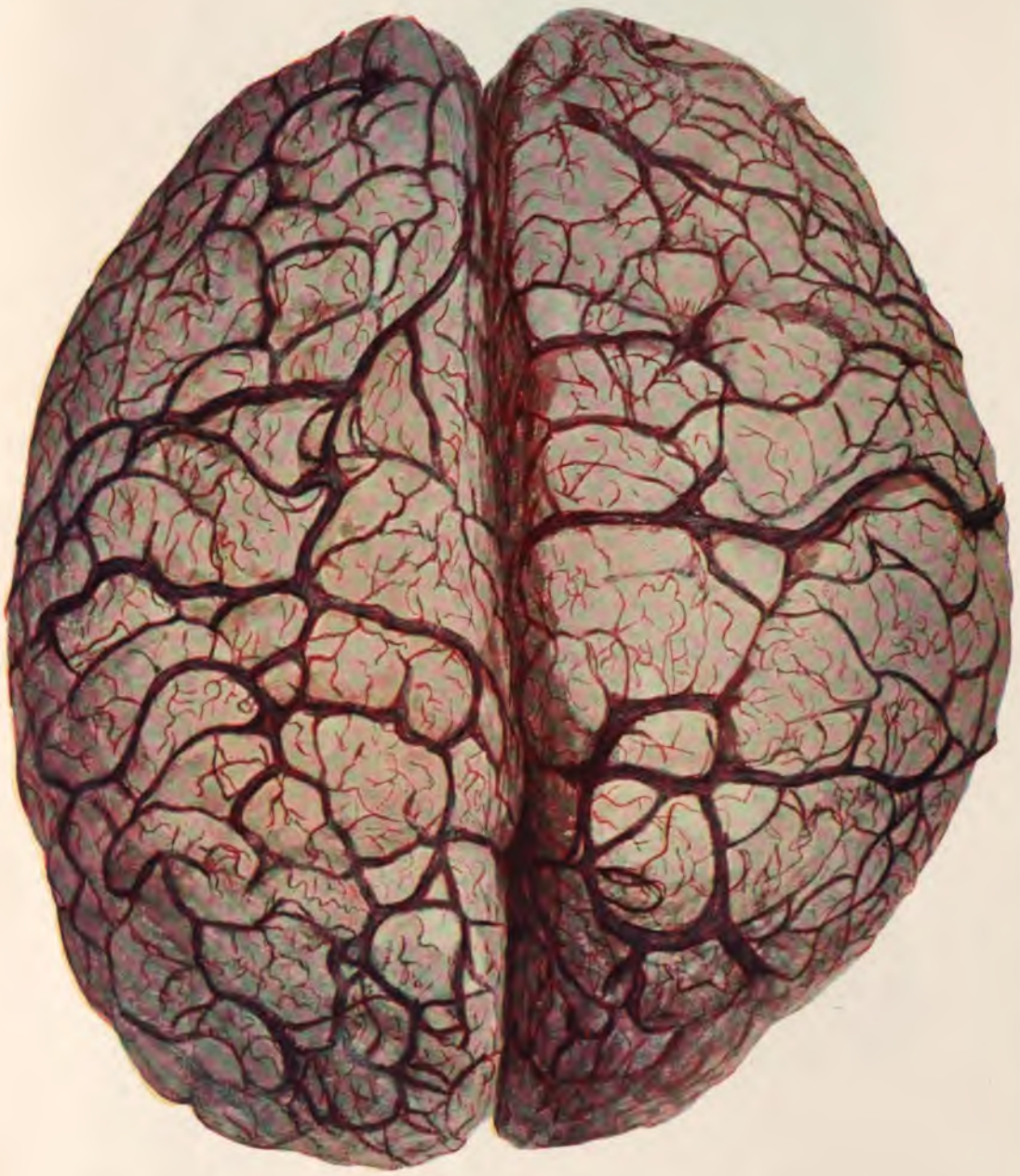
FIG. 1



Brain of a case of sleeping sickness (Kitambo). (See p. 66)

Fig. 2

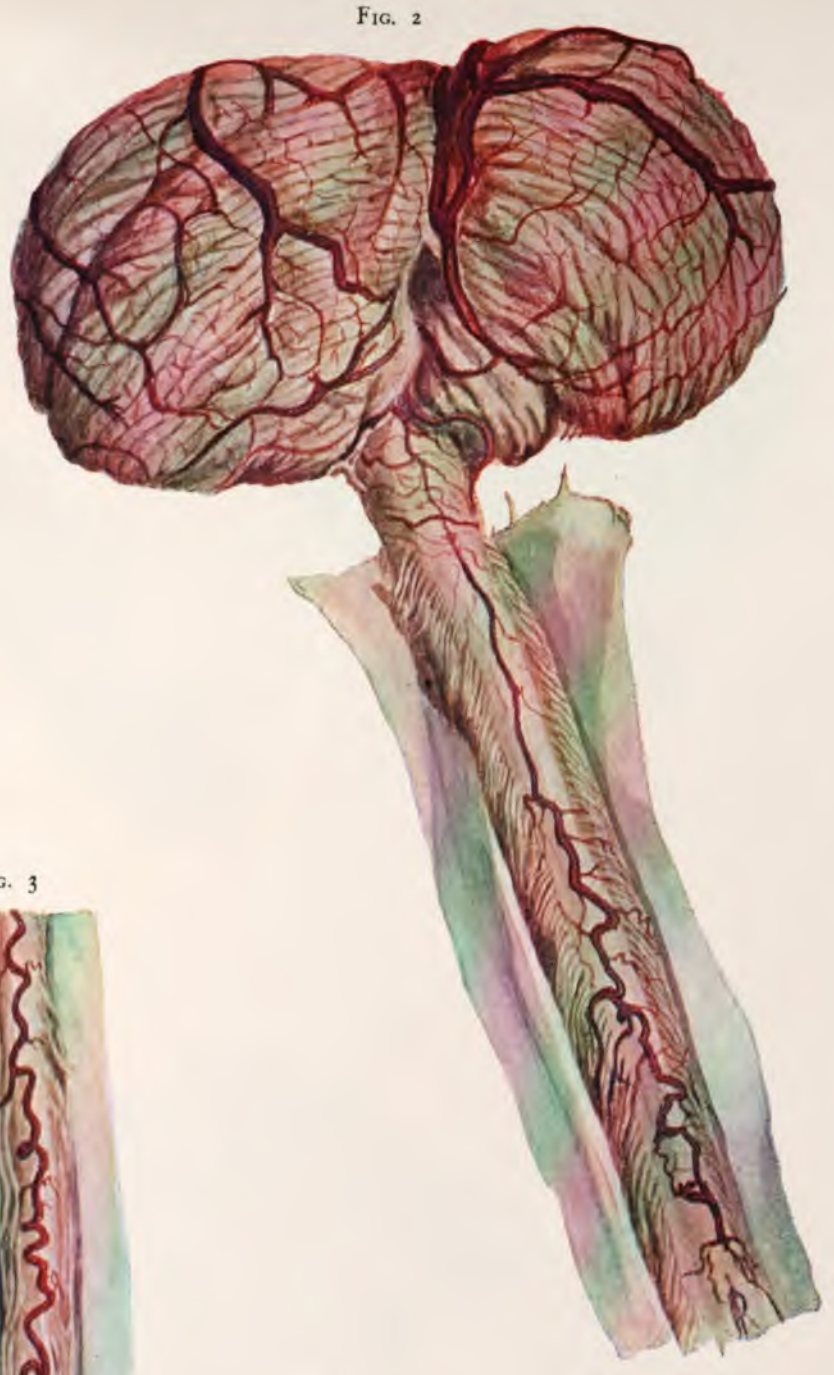
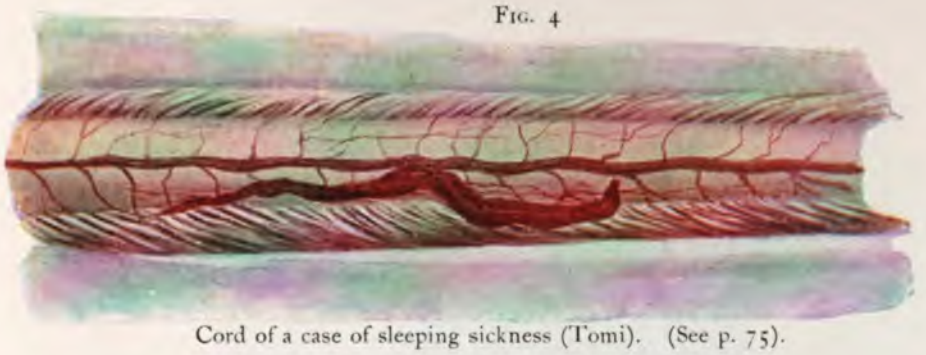


Fig. 3

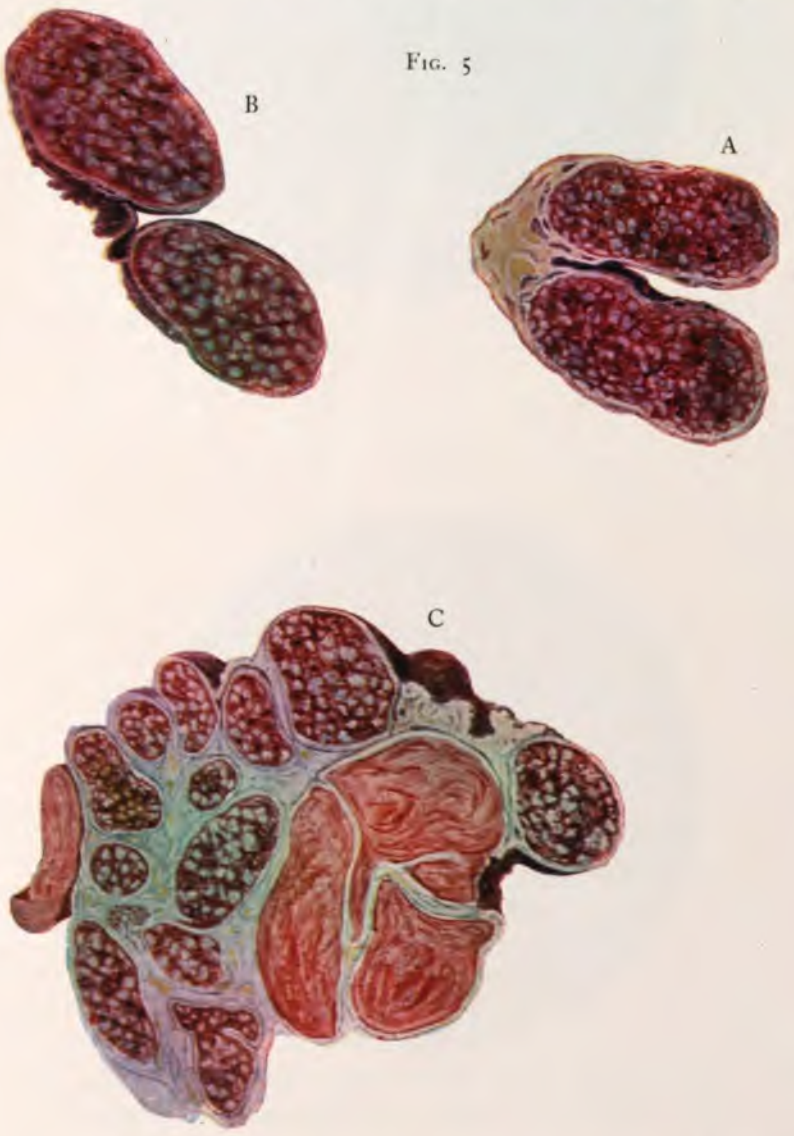
Cerebellum and cord of a case of sleeping sickness (Boyo). (See p. 81).

FIG. 4



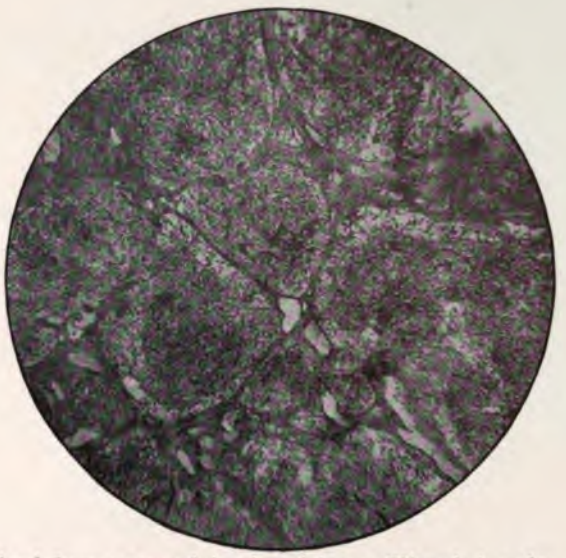
Cord of a case of sleeping sickness (Tomi). (See p. 75).

FIG. 5



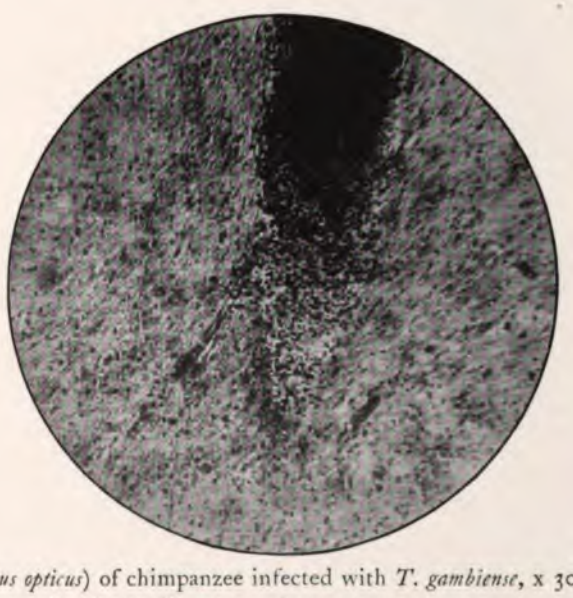
Lymphatic glands from a case of sleeping sickness (Tomi). (See p. 76).
To show hyperplasia of the connective tissue.
(A) and (B) Cervical ; (C) Retroperitoneal

FIG. 6



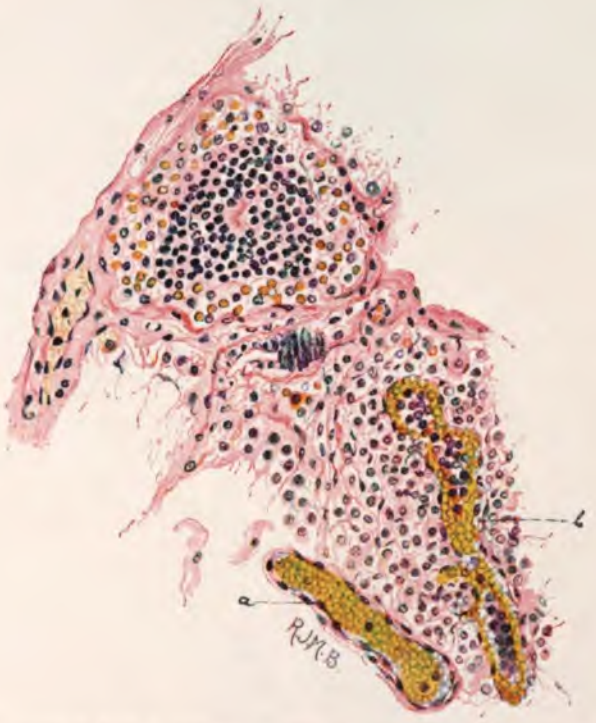
Section of cervical gland from a case of sleeping sickness (Kitambo), x 60. (See p. 70)
To show hyperplasia of connective tissue

FIG. 9



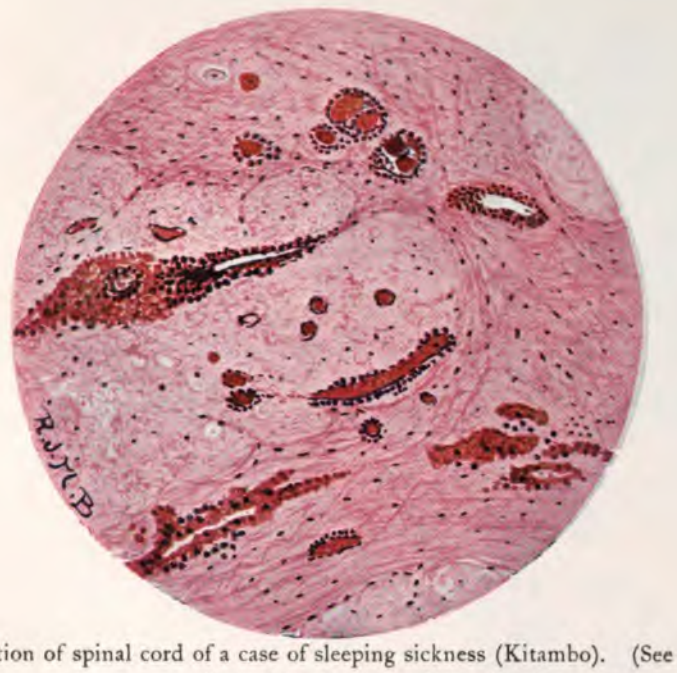
Section of brain (*Thalmus opticus*) of chimpanzee infected with *T. gambiense*, x 300. (See p. 85)
To show small-celled infiltration and haemorrhage around a vessel

FIG. 7



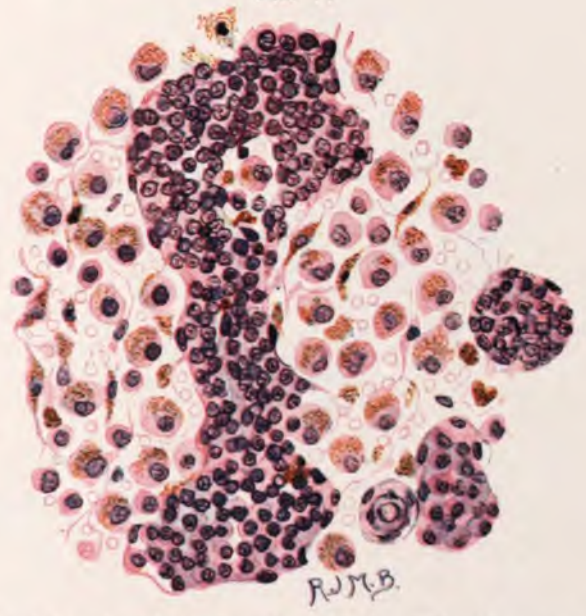
Section of mesenteric gland from a case of sleeping sickness (Kitambo), x 90. (See p. 70).
To show sinus formation. The sinus contains phagocytic cells and red blood corpuscles
(a) blood vessel ; (b) sinus

FIG. 8



Section of spinal cord of a case of sleeping sickness (Kitambo). (See p. 73).
Stained by haematoxylin and eosin

FIG. 11



Section of cervical gland of dog infected with *T. dimorphon*. (See p. 88.) Haematoxylin and
To show round-celled infiltration about vessels and numerous smaller and larger capillary haem

THE NATURE OF HUMAN TICK-FEVER IN THE EASTERN PART OF THE CONGO FREE STATE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE—MEMOIR XVII

THE NATURE OF HUMAN TICK-FEVER IN THE EASTERN PART OF THE CONGO FREE STATE

WITH

NOTES ON THE DISTRIBUTION AND BIONOMICS OF THE TICK

BY THE LATE

J. EVERETT DUTTON, M.B.

(WALTER MYERS FELLOW OF THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE)

AND

JOHN L. TODD, B.A., M.D. McGILL

AND AN APPENDIX ON THE EXTERNAL ANATOMY OF ORNITHODOROS MOUBATA

BY

ROBERT NEWSTEAD, A.L.S., F.E.S.

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PREFACE

Four months ago we were in possession of the main facts stated in this paper. Illness and death retarded the gathering of information and the recording of our observations. It is only now possible to publish a part of our work. At the end of November we both fell ill with recurrent fever. Dr. Dutton's illness was severe, and it was not until the middle of January that his convalescence obviously began. Unfortunately, he over-rated his strength, and even before his fever subsided commenced once more to work, harder and for longer hours than ever. On the twenty-first of February his fatal illness commenced, the twenty-seventh of February he died, after four days' unconsciousness.

For almost a year and a half Dr. Dutton and I have worked together in the Congo. This communication represents only a small part of the results of our observations. Other reports, based on our common work, must soon be written by me alone.

May any weakness in all be recognised as mine, and in nowise due to my absent comrade.

J. L. T.

KASONGO, March 28, 1905.*

* On March 24, 1905, we received the "British Medical Journal" for November 26, 1904, containing the note of Ross and Milne on tick-fever. They are to be congratulated on their discovery. The present paper will help to complete their observations.

THE NATURE OF HUMAN TICK-FEVER IN THE EASTERN PART OF THE CONGO FREE STATE

BY THE LATE
J. EVERETT DUTTON, M.B., VICT.
(WALTER MYERS FELLOW OF THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE)
AND
JOHN L. TODD, B.A., M.D McGILL.

On the twenty-sixth of November, 1904, we sent a message to the Committee of our School saying that a spirochaete was the pathogenic agent of the human tick-fever which occurs in the Oriental province of the Congo Free State, and that we had been able to infect monkeys with spirochaetes through the bites of ticks.

In the countries of Africa where it occurs the human tick is generally found to have an evil reputation among the natives. To its bite is attributed a sickness of longer or shorter duration, which may end in death. Accounts of the sickness are mainly derived from the writings of travellers.

Livingstone was the first (4, 5) to describe a "human tick disease" in Portuguese South Africa. In the beginning of 1903 Manson, in his work on Tropical Diseases, (1) was able to sum up in two short pages the known facts of the African disease.

Since our arrival in the Congo our attention has often been called to the human tick and to its evil properties. We found that natives who knew the arachnid had, as usual, a decided dread of it; but it was not until we had left Stanleyville, on our way up the Congo to Kasongo, that we constantly encountered the tick, and saw, for the first time, cases of the disease. We were at Nyangwé, an infective centre, from November 13 to 22, 1904. Here we collected a large number of ticks for experimental purposes, and were fortunate enough to obtain an autopsy on the only fatal case of "tick-fever" that we have seen. We reached Kasongo on November 23, and have here seen further cases, and have been ourselves attacked by the disease.

From this clinical material, from transmission experiments with ticks, and from information and reports received from residents in the Congo, we have been able to show that "tick-fever" in the Oriental province is a relapsing fever, produced by a spirochaete, probably identical with *Spirochaete Obermeieri*, and that this organism can be transmitted by the bite of the tick.*

In addition, we have in one experiment been successful in transmitting the spirillum by the bites of young ticks newly hatched in the laboratory from eggs laid by infected parents.

* We are not in a position to identify the species of ticks which we found in the Congo. All that we have seem to be the same as those we found in the Lower Congo, and judged to be *Ornithodoros moubata*. Some of our specimens have been taken home by Dr. Christy, and have been identified by Mr. R. I. Pocock, of the British Museum as *Ornithodoros moubata*.

The History of Human Tick-fever in the Oriental Province of the Congo Free State.

Livingstone mentions being annoyed by human ticks while at Nyangwé, in 1871 ; but he does not speak of them as producing disease (2, 3), although he was well acquainted with their pathogenic properties (4, 5). Dr. Hinde, who accompanied the expedition which drove the Arabs from this part of the Free State in 1892-94, saw, at Kasongo, ticks and sick persons who attributed their illness to the bites of these acarids (6). Although he lost some of his own men from the same cause, he believed that the tick was harmless, and the natives had died through the force of an ignorant superstition. His opinion has been shared by practically all the Europeans knowing this district whom we have met. Each told us of the "*Kimputu*"—the local name for the tick—and of the imagined deadliness of its bite. Almost no one believed that there might be some truth in the natives' belief. As a result, all who reported ill through "*Kimputu*" bites were at once suspected of malingering.

It is not altogether difficult to see how such a mistake could perpetuate itself. To a casual observer the human tick resembles the ordinary "harmless" cattle tick.

As Dr. Hinde justly observed (6), ills which have no connection with the tick are attributed by the natives to its bites. The real nature of tick-fever has probably escaped recognition by physicians—microscopes have not been used—through this fact, and because, as seems probable, atypical cases—such as those described below, "No. 7" and J. L. T."—may not infrequently occur. Among all the natives whom we have questioned, only one has certainly recognised the recurrent nature of tick-fever.

In the more typical cases the symptoms of a disease which prostrates the patient on one day and leaves him free from fever on the next must have puzzled many, who could not suspect their specific nature, were it not that to most persons in this part of Africa every fever is malarial, and quinine is at once given. The naturally falling temperature of relapsing fever might thus be easily made to furnish the "therapeutic proof" of its malarial nature. Our own experience illustrates how easily mistakes might occur in diagnoses uncontrolled by the microscope, and based on only a single observation made, perhaps, between the attacks. Since we have been in the Oriental province twenty-four reputed cases of tick-fever have come, or have been sent, to us. The symptoms of seven of these patients were explainable on other grounds. Six were infected with trypanosomata, one with malarial parasites. Seven of the remaining cases gave histories similar to that recorded below in Case 3, and no cause for their illness was discoverable during the one or two days that they were under our notice. In only ten did we find spirochaetes. We have in addition seen spirochaetes in two soldiers, new-comers, who had never heard of the "Kimputu." In all we have had under observation twelve natives infected with spirochaetes.

Immunity, Susceptibility, and Mortality.

Sir John Kirk said (1), while speaking of the tick-fever on the Zambiese, that after recovery from tick-fever the patient had complete immunity from further attacks. We have been told by natives that a man can only once be made ill by the "*Kimputu*." It seems very probable that those living in infected localities may have relapsing fever while young, and grow up immune to the affection. Several Zanzibar traders have lately told us that the human tick "*Papasi*" (7) of Udjiji and Zanzibar (they assert they are the same as the ticks of this region) are harmless, and that their bite gives no disease. As already noticed, Livingstone, in 1871, did not mention that the ticks—"*Tapasi*"—of the Arab town at Nyangwé produced any disease (3). Nyangwé has at present a particularly bad reputation. The natives living inland who are forced to come to that post always endeavour to avoid sleeping there. A small caravan comes monthly to Nyangwé to bring in produce from villages some two days' walk inland. The porters formerly slept at Nyangwé before returning home. As a result there was always some one sick with "*Kimputu*" in their villages. It is generally accepted that strangers are particularly susceptible, and it seems certain that the majority of cases in this district occur among newcomers. All of our cases, save Nos. 2, 4, 7, had recently been exposed, for, probably, the first time to tick bites.

The mortality from tick-fever does not seem to be very great amongst those who are cared for and not exposed to fatigue and hunger. Under adverse conditions the death rate may be high; for example, of twenty carriers who contracted the disease in one caravan sent from Kasongo to Kabambarré ten died

Inoubation Period.

The period intervening between the bite of the tick and the declaration of the disease is, from our experience, about one week. The natives usually put it at five days. Occasionally symptoms are said to follow within a few hours of the bite. In no case have we been able to personally verify the dates of both the bite and the commencement of the fever.

Symptomatology.

In the two European cases slight prodromata, mental heaviness, and lack of acuity were noted. In all the cases the onset of the fever has always been sudden, and in no instance preceded by a distinct rigor. The patient is prostrated, and complains chiefly of severe headache, usually frontal occasionally general. Boneache—in the limbs, and backache are distressing: the patient feels as if well beaten. There is a marked distaste for food. Vomiting generally occurs once or twice, but has never been continued. Slight diarrhoea is fairly constant, constipation may occur. The evening temperature during the attacks frequently reaches 104.5° F.; the highest temperature recorded is 105.3deg. F. There are usually three or four attacks which often end in more or less profuse perspiration. As a rule each attack lasts for three or four days, and the intervening periods vary from five to nineteen days.

The spleen is sometimes, not always, enlarged. Herpes, epistaxis, and hiccough, were complications observed. The most characteristic features of the disease have been the prostration of the patients during the attack of fever, and the quick return to comparative health with the fall of the temperature.

The following case reports, charts, and *post-mortem* finding certainly demonstrate the clinical identity of the tick-fever observed by us with the relapsing fever of the text-books (8, 9).

Reports of Native Cases.

CASE 1.—Female, age 27, seen at Lokandu, October 30, 1904, under observation for two days.

History:— Patient and her husband, a soldier, were on their way from Lake Tanganyika to the Lower Congo. On the night of October 12 she was bitten by ticks on the thigh, neck, and arm. Three ticks were caught in the morning, no obvious marks of the bites were left. The following day Kasongo was reached; here the patient remained for a week. On October 21, the day after leaving Kasongo, she fell sick. The incubation period, that is, the interval between the bites of the ticks and the appearance of symptoms, is, therefore, in this case about eight or nine days. On getting up in the morning she complained of terrible headache, accompanied by throbbing of the ears. Her husband says that there was fever but no shivering. The headache, though apparently chiefly frontal, extended around the head to the occiput. There was no accompanying nausea or vomiting.

Present Condition:—Temperature sub-normal; pulse 70; respiration 20. The patient is a fairly developed woman; no wasting. She has a pained expression, and keeps the head fixed. Because of severe headache has a piece of "tie-tie"—native cord—tied tightly around the head across the forehead. It is apparently disagreeable for her to be placed in a bright light. She has lost her appetite, and complains of a bad taste in the mouth. She walks gingerly with the help of a friend; extremities are cold; respirations somewhat laboured. Liver is not enlarged or tender. Spleen is distinctly enlarged, extends 5.5cm. below costal margin, not tender. Tongue is flabby and furred. Patient otherwise normal.

October 31st.—Temperature a.m., 97.8; p.m., 101.2; respiration and pulse as before. Headache is still very severe. A slight fulness around eyes is noticed.

Blood examination:—No malarial parasites or trypanosomes were seen in either fresh or stained preparations. In two stained films taken on October 30th two spirochaetes were seen.

CASE 2.—Female, age 26, seen at Ukungwa, October 31, 1904; under observation for one day.

History:—Patient was bitten a month ago by a tick. A week later high fever with chills commenced. At present has no fever, but is weak, quite thin, and still complains of severe headache and of pain in the eyes. Physical examination revealed nothing abnormal; the spleen was not enlarged. No malarial parasites or trypanosomes were seen in either fresh or stained preparations of blood. In four stained films as many spirochaetes were seen. Many ticks were found in the house—a rest-house for native travellers—in which this patient lived. She was the wife of the caretaker, and was not a native of the district.

CASE 3.—Male, age 20, seen November 6, 1904, at Sendwé; under observation for two days.

History:—Patient, a native of this neighbourhood had been employed as a labourer at Stanleyville. He was on his way home, and while at Ukungwa, where he slept in the house inhabited by Case 2, he was bitten by a tick. Patient says that symptoms

DISEASE.

Spirillar Fever

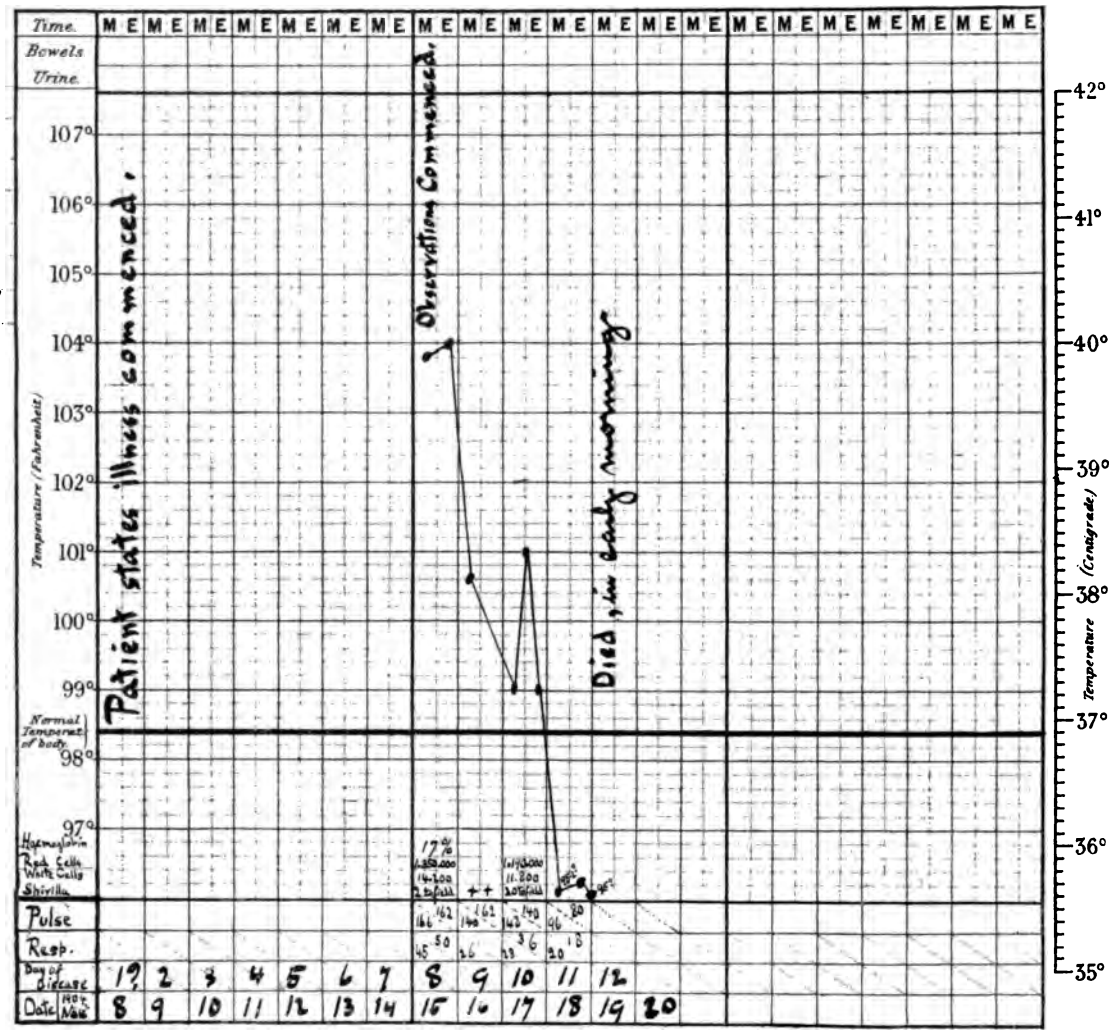
Notes of Case.

Name Case, 4.

Age 9, 14

African

Chart I



to face p 5

commenced on October 15th, four days after the bite. On his arrival at Sendwé, October 24th, where he was employed as a labourer, patient seemed strong and well, and it was not until about a week later (October 31) that he was noticed to be ill. It is very doubtful whether there was any rigor, and the history of fever during the first four or five days of the disease is uncertain. The patient says that his illness commenced with a "bad mouth," and a distaste for food. Pain in the eyes with frontal headache, pain in the nape of the neck and in the loins successively appeared. Patient asserts that these symptoms persisted, though lessening in intensity, up to the present (Nov. 6). There has been no vomiting or diarrhœa—took native enemata. The resident European says that patient is certainly thinner and less robust than on his arrival here.

Present Condition:—Pulse, respiration, and temperature normal. Patient is a thin man, has apparently lost flesh; is obviously weak; has pained expression; eyes watery. Physical examination showed nothing abnormal; spleen not enlarged or tender. The lymphatic glands of the inguinal region were alone enlarged. Blood examination:—Many fresh, centrifuged and stained preparations of the blood were taken. No malarial parasites, trypanosomata or spirochaetes were seen. A few filaria, sheathed and unsheathed, were present. Fresh and stained specimens of spleen juice showed no parasites. Gland juice was examined with negative result.

The history and record of examination of this patient are typical of six other cases who have been brought to us, some weeks after the commencement of their illness, but while they were still weak.

CASE 4 (*Chart No. 1*).—Female, age 14, seen at Nyangwé, November 15, 1904; under observation for four days preceding death.

History:—Patient was born and has always lived in this neighbourhood. On November 7, at about midnight, she was bitten on the foot by a tick. Before daybreak fever, headache, vomiting, and purging came on. The diarrhoea persisted for four days. The vomiting quickly stopped, and recurred only once on the third day of the disease. Though patient has always eaten well, she does not seem to have relished her food. On the second day of her illness she was brought to Nyangwé. She then had generalised headache and pain in her legs; she was in a fever, trembled continually and felt very weak. In spite of these symptoms she has continued work in the gardens.

Present Condition.—Temperature 103.8, pulse 166, respiration 45. Patient is a well-matured, but slight and thin child; is too weak to walk alone, and prefers to lie, not sit on the floor.

Although patient and her master deny that there has been any abeyance in the symptoms, it seems that she is more ill to-day (November 15) than yesterday. This morning she claims to have got up well and to have gone to work as usual, but as the sun became hot she became "tired."

Physical examination revealed no abnormalities save slight puffiness and yellowishness of face about the eyes, and a greatly enlarged spleen, which reached, patient lying on back, to below the umbilicus. Its superficial markings, in the recumbent position, are indicated in the accompanying photographs (Pl. 3, fig. 1).

Blood examination:—Coverslip preparations showed large numbers of spirilla. No malarial parasites or trypansomata were ever seen in this case. On splenic puncture the fluid aspirated contained spirilla in apparently the same numbers as finger blood.

The patient became steadily weaker, spirochaetes were constantly present in her blood, and she died early in the morning of November 19.

Autopsy:—The examination commenced at 9 a.m., six hours after death. The body was that of a thin, but not emaciated girl. Post mortem rigidity present throughout. Skin fairly clean, no eruption. Oedema of dorsa of both feet and of upper and lower eyelids. Pupils equally and widely dilated; mouth clean; no icterus. The usual incision was made; subcutaneous fat fair in amount; much light-coloured blood escapes from cut vessels—looks like blood-stained water; no oedema present over abdomen.

Abdomen: Contains about 5ccm. slightly blood-stained fluid; omentum retracted.

Thorax: Pericardium and pleura normal; about 50ccm. clear fluid in pericardial sac. Heart, weight 114 grammes, on whole fairly normal; muscle pale, firm, slight fatty change at apex of papillary muscle. The extended valves measure—pulmonary 5cm., tricuspid 9.26cm., mitral 8cm., aortic 4.75cm.

Lungs together weighed 342 grammes, are very pale, otherwise normal.

Liver: Weight 1,132 grammes, measures 23 by 14 by 8cm.; cuts firmly, on section very yellow and shiny; no amyloid reaction. Gall bladder pale, moderately filled with viscid bile.

Spleen: Weight 342 grammes, measures 15 by 8.75 by 5.5cm., cuts firm and hard, not easily friable.

Kidneys: Weight together 114 grammes, right measures 7.8 by 4.6 by 2.5cm., cortex 0.6cm., both are almost bloodless and cut firmly, capsule peels with ease; colour, particularly in pyramids, is dirty, brownish-yellow, suggesting fatty degeneration.

Bladder: Normal, filled with highly coloured urine.

Pancreas normal. Stomach and intestines contained no food, many round worms, and very many anchylostomes.

Lymphatic glands normal. Bone marrow at middle of femur was saffron-coloured and firm.

Films taken at the autopsy showed the continued presence of spirochaetes.

CASE 5.—Female, age 28; under partial observation during course of illness.

History:—The patient, coming from Stanleyville, arrived at Nyangwé on November 13. The illness commenced there on November 22 with fever, headache, and pain in the limbs. There was no history of a tick bite. On November 23, fever and pain persist; severe vomiting. November 25, temperature 99.6, feels almost well, complains bitterly of aching thighs and calves. Patient remained well until December 4, when the symptoms recurred and spirilla were found in the blood (first examination). This, the second attack, lasted until December 8. There was then a complete absence of symptoms until December 17, when the third and last attack came on with severe vomiting, boneache, and fever.

CASE 6.—Female, age 18; under partial observation during course of illness.

History:—Patient, coming from Stanleyville, arrived at Nyangwé on November 13. The illness commenced there on November 21 with fever, very severe headache, vomiting (once), diarrhoea, and pain in the back. There is no history of a tick bite. On November 22 and 23 the temperature varied between 104.5 at night and 101 in the morning. On November 24 temperature normal, and patient, save weakness, well. Second attack commenced on November 29, with the same symptoms, evening temperature 104. On the afternoon of November 30 profuse perspiration came on, and the temperature fell to normal. Patient then remained without fever until December 14 and 15, when the usual symptoms returned, evening temperature 103. December 16, temperature returned to normal, and patient remained well.

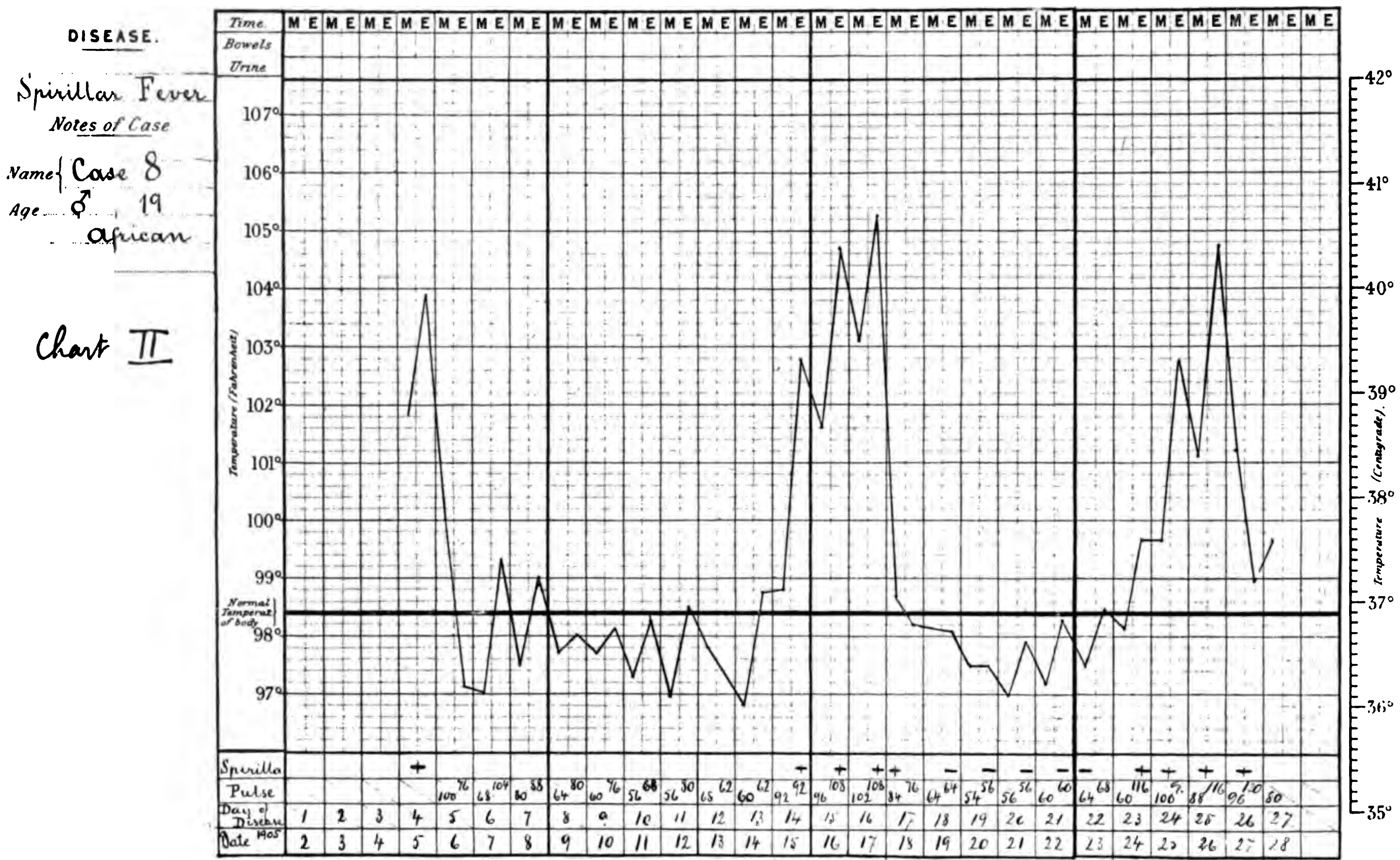
Rather profuse expistaxis occurred in this case on November 22 and 29. The spleen, slightly enlarged after the first attack, was, after the third, 5cm. below the costal margin. The blood was examined on November 29, and numerous spirochaetes seen.

CASE 7.—Male, age 26; seen January 6, at Kasongo; under observation for five weeks.

History:—Patient has lived in Kasongo for the past two years. He states that about a week or ten days ago he was bitten during the night by two ticks. He caught them, burned them, cut his skin at the site of the bites, and rubbed in the ashes as a preventive against tick fever.*

Two days ago his illness commenced, he complained of feeling cold, having fever, trembling and weakness. He has been unable to take food for two days.

* This is the treatment usually employed by natives of this district for tick bites. Its prophylactic powers are widely believed in. Livingstone mentions (5) an analogous practice.


DISEASE.
Spirillar Fever
Notes of Case
Name { Case 8
Age ♂ 19
African
Chart II
Time
Bowels
Urine
Temperature (Fahrenheit)
107° 106° 105° 104° 103° 102° 101° 100° 99° 98° 97°
Normal Temperat. of body
Temperature (Centigrade)
42° 41° 40° 39° 38° 37° 36° 35°
Spirilla
Pulse
Day of Disease
Date 1905

to face p. 7

DISEASE.

Spirillar Fever

Notes of Case.

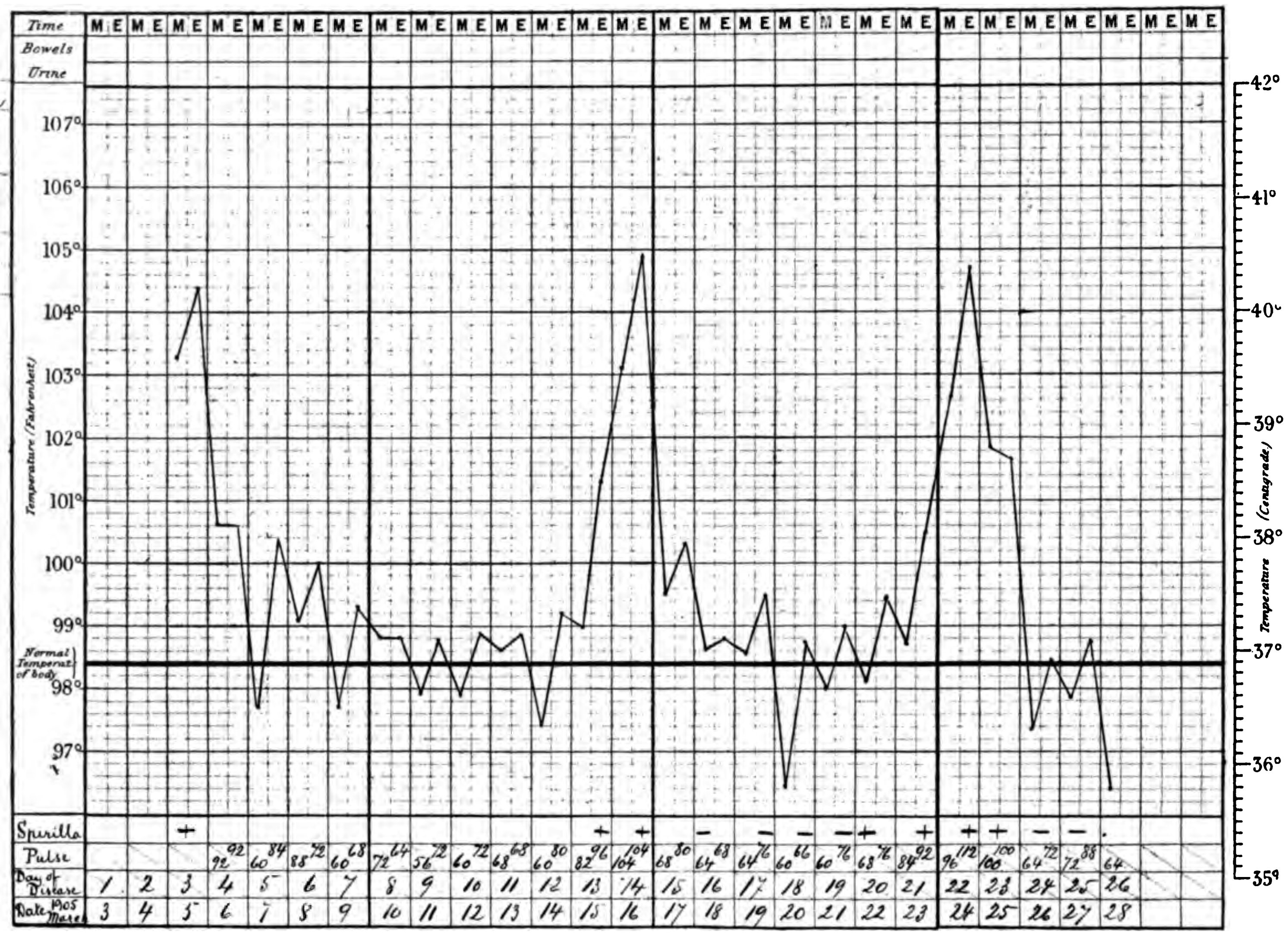
Name {Case 9

Age 20 ♂

African

Case Book No

Chart III



p. 7

DISEASE.

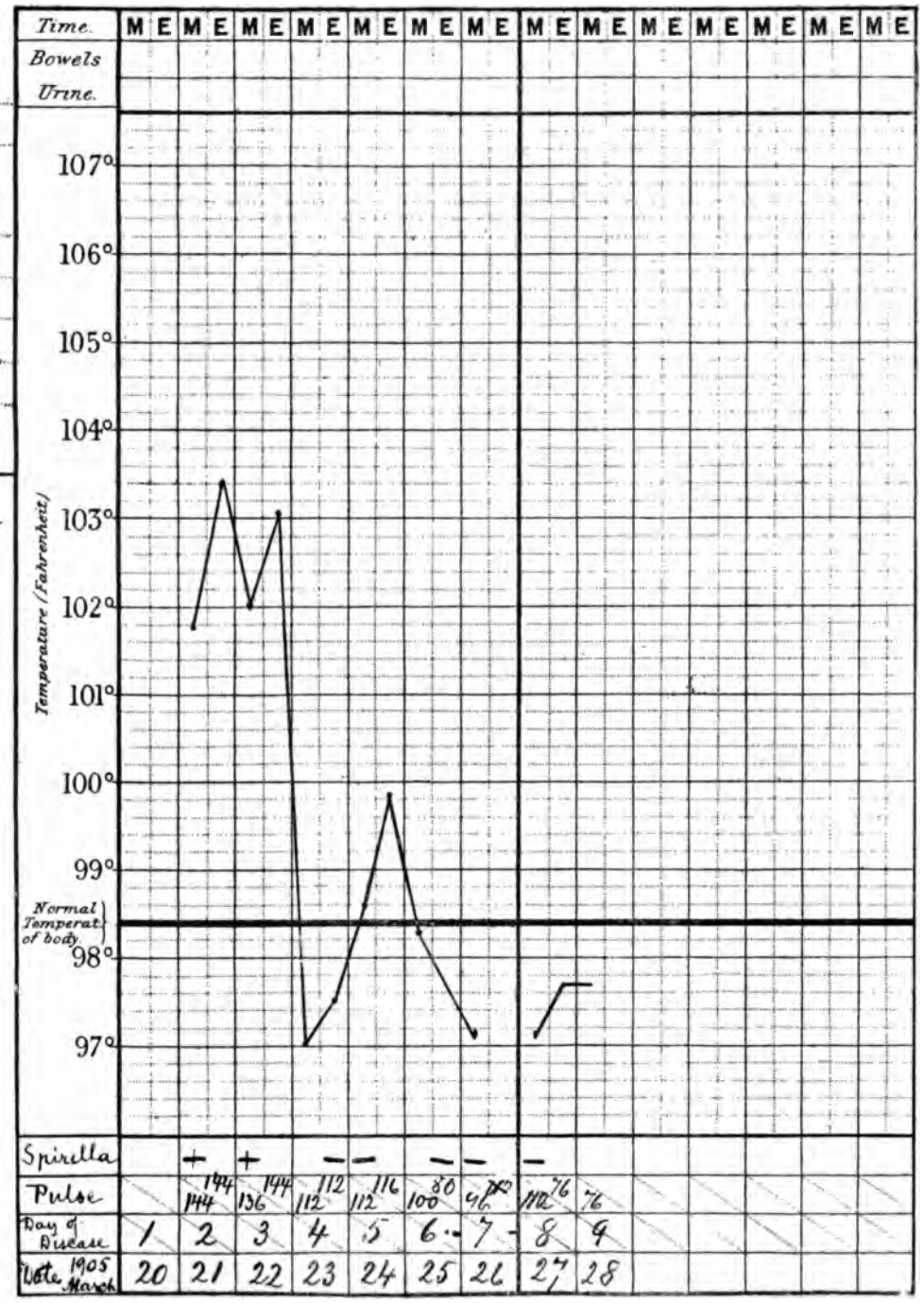
Spirillar Fever

Notes of Case.

Name { Case 10.
5, ♂
African

N°

Chart IV



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to face p. 7

DISEASE.

Spirillar Fever

Notes of Case.

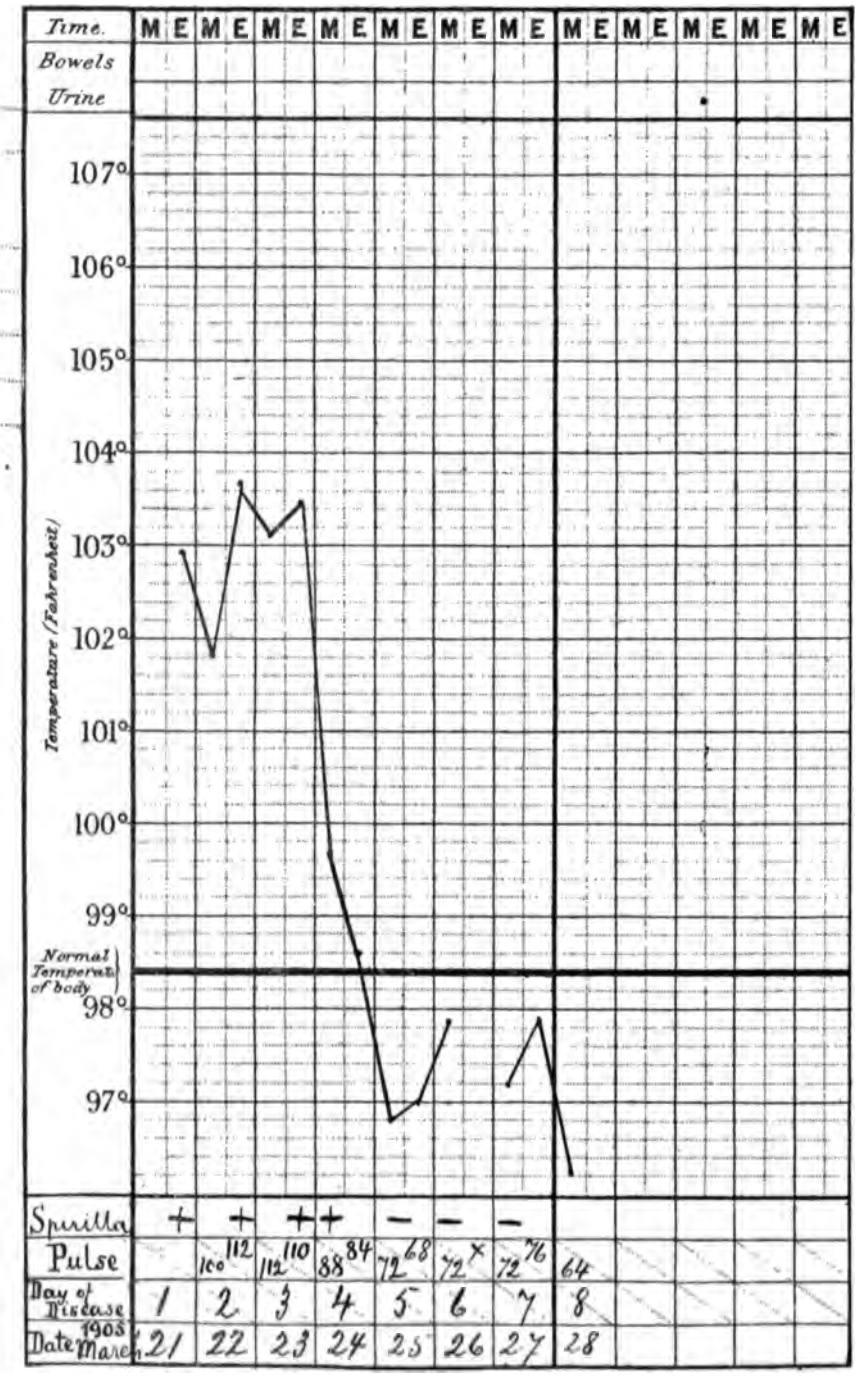
Name { Case 11

Age 28 ♀

African

Mother of case 10.

Chart V



Spirilla	+	+	+	+	−	−	−								
Pulse		100 112	112 110	88 84	72 68	72 ×	72 76	64							
Day of Disease	1	2	3	4	5	6	7	8							
Date 1903 March	21	22	23	24	25	26	27	28							

× absent

Physical examination: Temperature 101.4, revealed nothing abnormal, spleen not palpable. Spirochaetes, not numerous, were seen in fresh and stained specimens of the blood by Dr. Heiberg. In spite of repeated examinations, spirilla were never again seen in the blood of this patient, although on January 11 and 13 there was a well-marked rise of temperature (highest 102), and again between January 20 and 31 there was constantly a slight (99 to 100) evening temperature to be recorded.

CASE 8 (*Chart No. 2*).—Male, age 19, first seen March 5, still under observation.

History:—Patient is a soldier, one of a detachment on their way from the Lower Congo to Lake Kivu. He slept on the night between the 23rd and 24th of February at Nyangwé. He fell sick on March 2, at Kasongo, with frontal headache, pain in the back and limbs, anorexia, vomiting, continued constipation, and fever. The liver was normal; the spleen slightly enlarged and tender. The course of the disease was uneventful. The results of blood examinations are entered on the chart.

CASE 9 (*Chart No. 3*).—Male, age 20; first seen March 5, still under observation.

History:—Patient belongs to the same detachment of soldiers as Case 8. The history and symptoms were the same in both, save that the illness commenced in this case on March 3, and there was no vomiting.

The temperature curves of these two cases are remarkably similar.

CASE 10 (*Chart No. 4*).—Female, age 5; first seen on March 21, 1905, still under observation.

The patient is the daughter of a soldier on his way from the north of the Lake Tanganyika to the Lower Congo. She was bitten near Kabambarré on the night between the 13th and 14th of March. The illness commenced on March 20 with headache, pain in all the body, restlessness, anorexia, no vomiting, constipation, and fever. Liver was not enlarged. At the first examination the spleen was found to extend to 8cm. below the costal margin. On March 25 and 26 the pulse was irregular. No malarial parasites have ever been seen in this case.

CASE 11 (*Chart No. 5*).—Female, age 28; first seen on March 21, 1905; still under observation.

Patient is the mother of the preceding case, and was bitten at the same time. Her illness commenced on March 21 with the usual symptoms, the headache being distinctly temporal. There was no vomiting, and the spleen was never enlarged.

CASE 12.—Female, age 27; seen first on March 21; still under observation.

Patient is the wife of a soldier on his way from the Lower Congo to Lake Tanganyika. There is no history of tick bite. Illness commenced March 19 with general malaise, pain in forehead and nape of neck, anorexia — but no vomiting, constipation. Liver normal. Spleen enlarged and tender. Blood was examined on March 21, and spirilla found. The temperature on March 21 was 100.4; it has since been normal, and parasites have not been again seen.

Reports of European Cases.

J. E. D. (*Chart No. 6*). (*Patient's Own Notes.*)

Nov. 25.—This evening felt tired and unable to work as usual; have been partially constipated for past four days.

Nov. 26.—This morning was unable to take breakfast—loathing for food—nevertheless, swallowed a little bread and tea, and started at eight o'clock, after the sun had well risen, on a seven mile walk to Vieux Kasongo. During the first hour felt fairly "fit," and refused to ride on mule. As the sun became warmer, commenced to feel uncomfortable, arms ached so that it was almost impossible to hold sun-umbrella, and reached

White Fathers' Mission at the sixth mile in almost a collapsed state. Was carried in hammock to destination. Blood was examined at 4 p.m., and nothing seen. Six grains of calomel taken, and two small motions obtained. During the night was much distressed by fulness of the abdomen, and vomited four times. A few drops *Tr. Opii.* gave much relief.

Nov. 27.—Felt very slightly better in early morning. Head felt as if "muffled up," and severe throbbing, frontal aching soon developed. Had slight pains in small of back. Fulness and distention of abdomen as distressing as ever. Four cathartic pills taken in the morning had no effect, so calomel grs. 6 was taken, and several small liquid stools were passed during the night. Blood was examined at nine this morning and spirochaetes found.

Nov. 28.—Passed a bad night (7½ grains of antipyrine taken at 1 a.m.), because of severe throbbing headache and distressing fulness of abdomen; vomited twice during the night. Tried to get up in early morning, but soon returned to bed because of fever, weakness, and exhaustion; headache not so bad. Blood examined, spirochaetes present. Antipyrine grs. 10 taken at noon and saline laxative in the afternoon.

Nov. 29.—Was awake, up and down, all night, but not so distressed as during two preceding nights. Started in early morning, carried in hammock, for Kasongo, where arrived at 8 a.m. Throughout this access of fever a constant soapy taste was noted in the saliva.

Nov. 30.—Much better, went for walk; noted bodily weakness.

Dec. 1.—Passed fair night. Woke up after after-dinner nap with distinct oedema around eyes. Herpes has formed on upper lip and just within right nostril; there are two small ulcers on gums.

Dec. 2 to 6.—Save for slight looseness of bowels absolutely well.

Dec. 7.—Slight weariness in arms and legs; on coming in from morning bicycle ride felt shivery.

Dec. 8.—In bed, same symptoms as in previous attack—headache, boneache, lassitude, distaste for food—but no vomiting. Had no great thirst.

Dec. 9.—Same condition, constipated.

Dec. 10.—During early morning temperature fell to 97—a slight perspiration preceding—headache disappeared and appetite returned.

Dec. 11-15.—Fairly well and able to work.

Dec. 16.—Fever recurs in usual manner.

Dec. 17.—Headache bad; 7½ grains of antipyrine at noon. Temperature fell to normal after tepid bath at 10 p.m.

Dec. 18.—Weak, bowels loose, otherwise well.

For the following week (Dec. 19-25) felt very well, appetite was good. From Dec. 26 had a constant slight evening temperature, was rather bilious and constipated (calomel grs. 5, Dec. 31), and had a slight localised boneache, especially in the fore-arms.

Jan. 7.—Felt chilly and good for nothing this evening.

Jan. 8, 9, and 10.—Recurrence of fever with same symptoms as before. The temperature suddenly fell on the night of the 9th during a second heavy sweat. There was no further recurrence of fever. After each attack there was slight looseness of bowels and increase in amount and frequency of urine. The slight ulcer in the nostril lasted for some time after the labial herpes had disappeared. The spleen was tender, but was never easily palpable. For a fortnight succeeding the last attack occasional small evening rises of temperature with slight malaise were recorded. The appetite and general sense of well-being quickly improved under arsenic. Good health continued until about February 15.*.

The temperature chart and diary of this case record graphically the classical description of relapsing fever (8, 9). Spirilla were seen in the blood during each access of fever. Malarial parasites were always absent, and the patient has never at any time had malarial fever.

* No spirochaetes were seen during J. L. D.'s fatal illness.

DISEASE.

Spirillar Fever

Notes of Case

Name J. T. C.

Age 28 ♂

European

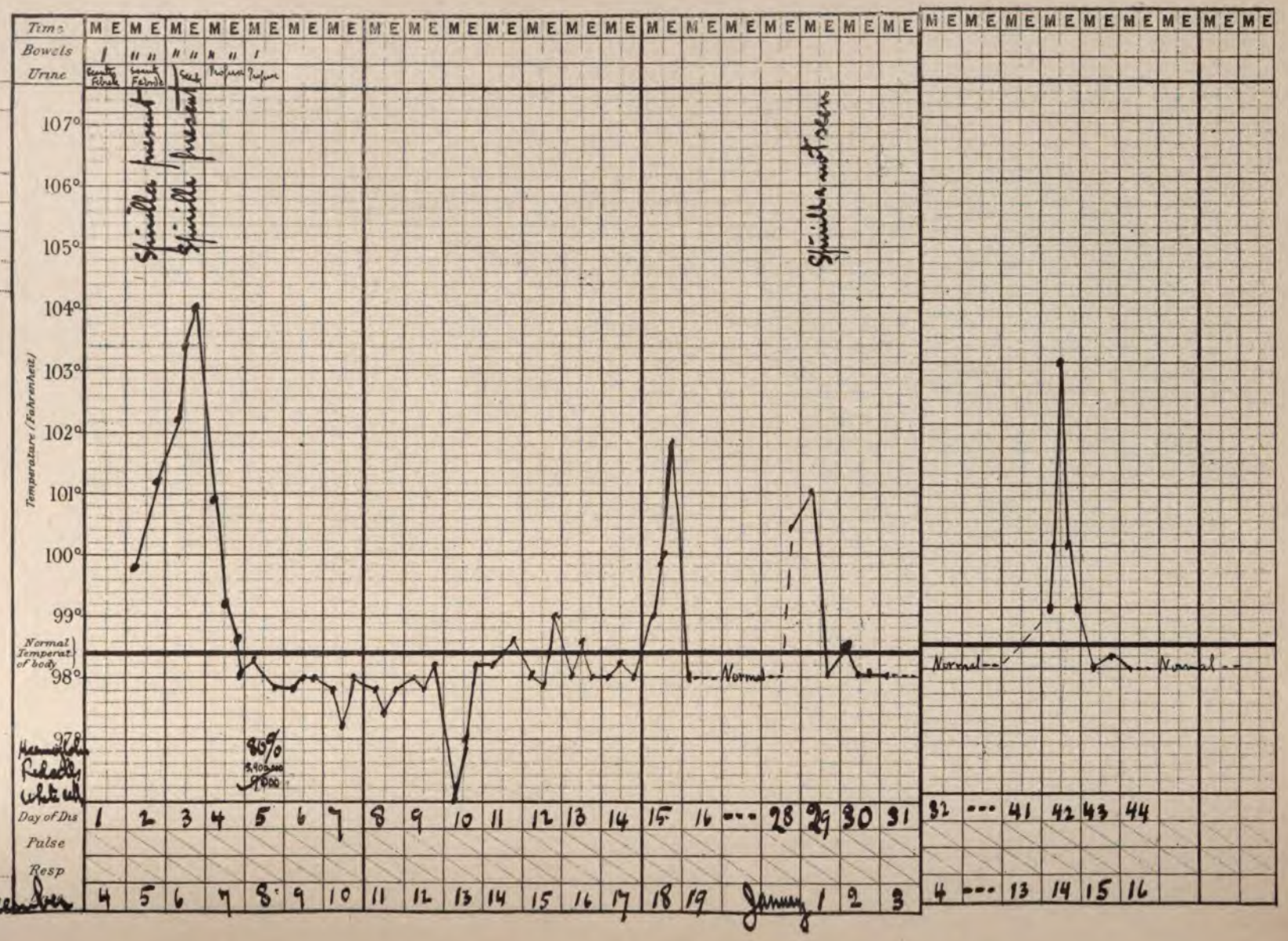
Case Book N°

Chart VII

Date of admission

Result

to face p. 9



The disease was most probably contracted at the autopsy done on Case 4, at Nyangwé, on November 19; after the autopsy was finished a slight abrasion was noted at the side of one of the finger nails. If such is the case the incubation period was, as usual (8), seven days. Though there is no knowledge of a tick-bite, the possibility of infection by that means cannot be definitely excluded, since on arriving at Kasongo ticks—two—were found in an unopened valise and in a bed-covering in daily use; and this although no ticks were seen in the rooms we had occupied in a few days before at Nyangwé.

The second European case was much the less severe. The symptoms—head and boneache, lassitude, slight nausea and diarrhoea, scanty febrile urine during and increasing micturition after the attack, tenderness over the unenlarged spleen, and fever noted in the first case were all present in the second, but with greatly lessened intensity.

The diary of the case is as follows:—

J. L. T. (*Chart No. 7*). (*Patient's Own Notes.*)

Dec. 3 and 4.—Felt vaguely unwell. Quinine 7½ grains each day.

Dec. 5 and 6.—Quite ill; blood examined, spirochaetes present—no malarial parasites seen.

Dec. 7 to 17.—Felt perfectly well. Quinine grs. 5 taken on Dec. 8 and 9.

Dec. 18.—Only slight malaise; small epistaxis during night. Blood not examined.

Dec. 19 to 31.—Well.

Jan. 1.—Passed a bad night; head and backache annoying. For three or four succeeding days was much troubled with neuralgia (patient not neuralgic). Blood examined, spirochaetes present.

Jan. 5 to 15.—Well.

Jan. 13.—Passed an uneasy night, slight diarrhoea.

Jan. 14.—Usual symptoms; great feeling of weakness.

Jan. 15 to present.—Patient free from symptoms, and entirely well. No malarial parasites were seen during the whole of this illness.

The Disease in Experimental Animals.

As the following abstracts of experiments indicate, the spirochaete does not kill the ordinary laboratory animals. To monkeys alone, the *Cercopitheci*, especially young animals, does it seem uniformly pathogenic. An adult rabbit (Ex. 156) was refractile. A young rabbit (Ex. 154) and a rat (Ex. 162), in whom there were grave accompanying affections, seemed more susceptible. A large guinea pig (Ex. 155) showed spirochaetes in its blood for only two days succeeding the inoculation of a large dose of heavily infected blood. In it, as in three rats (Ex. 163, 166, and 185), from whose blood spirochaetes, once present, finally disappeared, there appears to have been a temporary increase in the number of the parasites, and dividing forms were seen.

RABBITS.

Ex. 154: Rabbit, about one month old, weight 675 grammes.

Nov. 15.—Inoculated with about 5cm. blood from Case 4, showing two spirochaetes to field.

Nov. 16 to 17.—Scanty parasites present in blood. No spirochaetes were then seen until Nov. 24 and 25, when there were about twenty to a field (1-12th oil immersion objective; No. 4, ocular; Zeiss). In spite of daily examination no parasites were again seen. The animal died in convulsions on Jan. 23. The probable immediate cause of death was a very severe skin disease (?).

Preparations of blood and bone-marrow made at autopsy showed no parasites. Organs appeared normal.

Ex. 156: Adult rabbit, weight 2,497 grammes.

Much over 500 ticks, many of them certainly infected, have fed upon this animal. On Dec. 10 it was inoculated with blood containing numerous spirochaetes. Its blood was examined daily up to the end of December, and occasionally since. Parasites have never been seen.

GUINEA PIGS.

Ex. 155: Guinea pig, weight 453 grammes.

Was inoculated on Nov. 15, Nov. 17, Dec. 2, Dec. 10, and Dec. 17 with small quantities of blood containing spirochaetes, taken from patients or experimental animals. Daily examinations were made, but parasites were never seen. On Jan. 23, 4ccm. of blood, showing one spirillum to field (as before 1-12th immersion and No. 4 ocular), taken from an infected monkey, was inoculated. Parasites in very small numbers were seen during the two succeeding days and not again.

RATS.

Ex. 162: Adult rat, weight 266 grammes.

Dec. 5 to 12.—Twelve of the ticks which infected Ex. 157 were repeatedly fed on this animal. Dec. 13.—Spirochaetes seen in blood. Parasites were continually present until Dec. 19, when animal died. Death was undoubtedly largely due to a large, traumatic, subcutaneous abscess.

Ex. 163: Young rat, weight 65 grammes.

Dec. 10.—Inoculated with 3ccm. blood, containing numerous spirochaetes taken from an infected monkey. The blood was examined daily until Dec. 29. Parasites were seen in large numbers only on Dec. 12, 13, 14, 17, and 19 (not examined on Dec. 11).

Ex. 166: Adult rat, weight 168 grammes.

From Dec. 19 to 31, the ticks used in Ex. 162 (see above) fed repeatedly. The rat did not become infected. On Jan. 23 it was therefore inoculated with 3.5ccm. of blood, containing numerous spirilla, taken from an infected monkey. The blood was examined daily until Feb. 1, and since then at intervals of a few days. Very scanty parasites were seen only on Jan. 26, 27, 30, and Feb. 1.

Ex. 185: Rat, young, weight 91 grammes.

Jan. 23.—Inoculated with 2.5ccm. blood, containing many spirochaetes, from an infected monkey. Blood was examined daily until Feb. 1, and at intervals since. Parasites were seen on Jan. 24, 25, and 26.

MONKEYS.

We have been able to infect monkeys, five *Cercopithecus* (?*Schmidti*), one *Cercopithecus campbelli*, and one *Cercopithecus* (Sp. ?), with spirochaetes by the bites of ticks. Each animal was examined with negative result before the commencement of the experiment. Thirty other monkeys of the same species, caught in the same districts, and kept under the same conditions have been used for other experiments. Their blood has been constantly examined, and none have ever been found to be infected with spirochaetes.

Ex. 157: Young Cercopithecus, weight 1,586 grammes. (Chart No. 8.)

Nov. 19, 20, and 21.—Ticks (231 in all), caught in native houses at Nyangwé were allowed to thoroughly feed on this animal. On Nov. 24 spirochaetes were detected in its blood. The parasites later increased greatly in number. There was a high temperature. The monkey became very thin and weak. Anæmia was very pronounced, and death occurred on Dec. 4, after two days of utter prostration, during which the monkey scarcely moved, but remained dozing, crouched on his haunches. The autopsy was done immediately after death. Blood was very pale, and all organs anæmic. Long bone-

DISEASE.

Spirilla

Notes of Case.

Name { Ex. 157

Age

Diet young Monkey

Case Book N°

[Cercopithecus

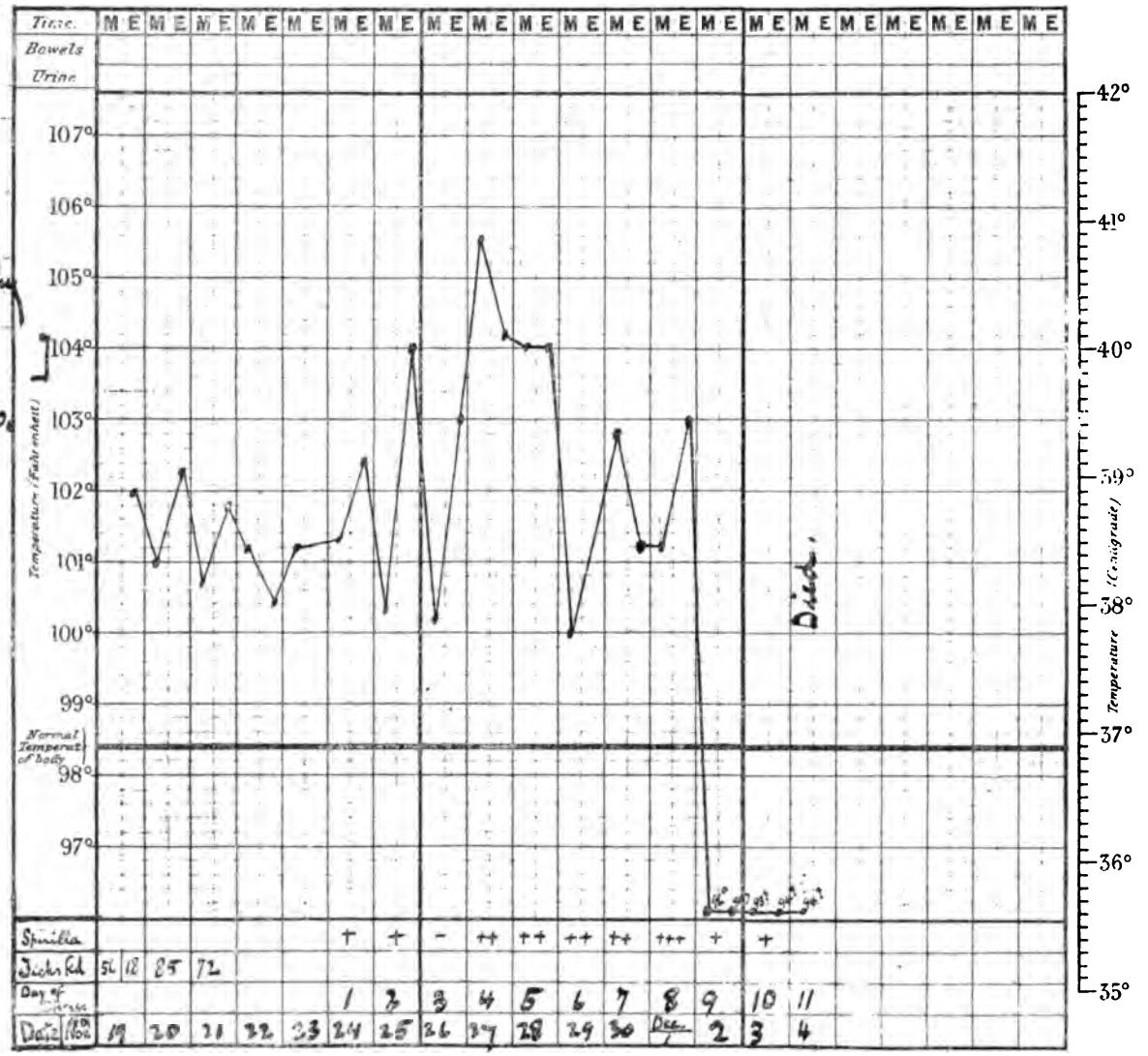
weight 3½ lbs

No malaria

No trypanosomes

No filariae

Chart VIII



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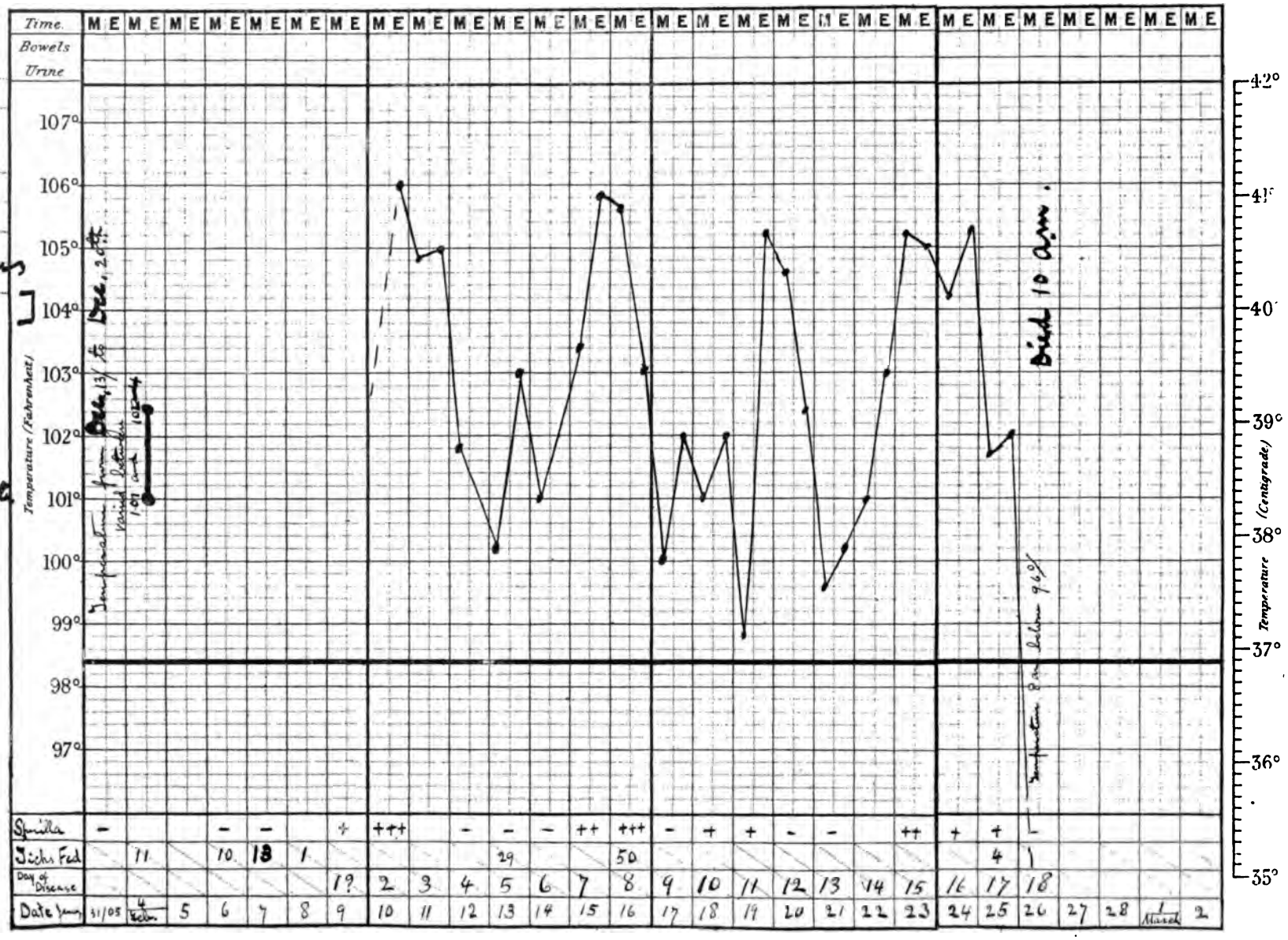
DISEASE.
Spirilla

Notes of Case

Name { Et. 192.
Age Laundress
Diet Cercopithecus
Case Book N°

Chart IX

No malaria
No trypanosomata
No filariae



to face p. 11

marrow was diffluent and chocolate coloured. Heart, kidney, and liver seemed cloudy. Spleen, enlarged, measured 7 by 2.8 by 1.7cm.; firm. No malarial parasites, trypanosomata, or filariae were seen in this animal.

Ex. 160: Young Cercopithecus, weight 1,586 grammes.

Ticks caught in native houses at Nyangwé were, on Nov. 24, fed upon a small rabbit (Ex. 154), whose blood contained many spirochaetes. On Nov. 30 twenty-six of these ticks fed upon this monkey. On the morning of Dec. 5 spirochaetes were for the first time seen in its blood. The parasites became more numerous, and were constantly present. On Dec. 10 the temperature was sub-normal, and the animal dying. It was, therefore, killed to obtain blood for inoculating exps. 155, 156, 163. No malarial parasites, trypanosomata, or filariae were ever seen in this monkey.

Ex. 167: Young Cercopithecus, weight 906 grammes.

Dec. 21 and 22.—One hundred and seventeen ticks caught in native houses at Nyangwé before Nov. 23, and not since fed, were allowed to feed upon this monkey. On Dec. 28 spirochaetes were seen in the blood for the first time, and the temperature rose. Parasites were constantly present, at first in large numbers, until Jan. 10. From that date until death on Jan. 19 parasites were absent from the peripheral blood—examined both during the day and at night. During the last few days the animal was extremely weak, hardly able to crawl, and had slight diarrhoea. The autopsy was commenced immediately after death. The animal was very thin, weight 604 grammes, all fat had disappeared. Spleen was slightly enlarged, 3.25 by 1.75cm., dark and firm. All other organs were extremely anæmic. Long bone-marrow dark red. A few spirochaetes were seen in preparations of heart blood, bone-marrow, and spleen juice taken at the autopsy. No malarial parasites, trypanosomata, or filariae were ever seen in this animal.

Ex. 176: Young Cercopithecus, weight 1,020 grammes.

On Jan. 12 and 13 258 ticks, caught at Nyangwé, before Nov. 23, and not since fed, were allowed to feed upon this monkey. On Jan. 16, 76, and on Jan. 19, 240 of the same ticks were re-fed. On Jan. 21 spirochaetes in fair numbers, were seen in the blood for the first time. The animal died on Jan. 23. Death was largely due to rough handling while feeding further batches of ticks. Spleen was slightly enlarged, 4.75 by 3cm., dark and firm. This monkey was infected with malaria, but neither filariae nor trypanosomata were seen in its blood.

Ex. 184: Adult Cercopithecus, weight 2,380 grammes.

On January 23, the ticks which had infected Ex. 160 on Nov. 30, and had since repeatedly fed on uninfected animals, were placed on this monkey; thirteen fed. On Jan. 28, Feb. 1, 2, and 6, they had again opportunities of feeding, the monkey being bitten fifty-nine times. On Feb. 6 another lot of infected ticks were accidentally placed on this animal for a few minutes. Three or four may have punctured the skin, none fed. On Feb. 14 and 18 the monkey was bitten twenty-three times by the first lot of ticks. On Feb. 19 spirochaetes were seen in the blood for the first time, and the temperature rose. Parasites were fairly constantly present in the peripheral blood until March 7. Since then they have been absent, though the temperature remains high. The animal is still under observation.*

Ex. 192 (Chart 9): Young Cercopithecus, weight 1,020 grammes.

It was thought possible that "J. L. T." might have become infected with spirilla through his unprotected hands while doing the autopsy on Case 4. To ascertain whether a susceptible animal would become infected with spirochaetes if parasite-containing blood were placed on its unbroken skin, the hair was carefully clipped from the abdomen of this monkey, a healthy area was chosen, and, after a careful negative examination—with a lens—for cuts and abrasions, a few drops of blood containing many spirochaetes—from Exps. 162 and 163—were placed upon the unbroken skin. The blood was covered with a damp chamber to prevent its drying, and was removed after thirty minutes. This was done on Dec. 13, 15, and 17. The blood was examined daily until Dec. 27. As no parasites were seen, the animal was thought to be uninfected, and it was decided to use it for a second experiment.

* October 23rd, 1905. Temperature constantly elevated (malaria). Blood frequently examined; no spirochaetes seen until May 13th, 1905 (T. 107.2) one to field. None were again seen, though blood was constantly examined, and on August 23rd the animal died in convulsions (no spirochaetes found at autopsy). ? Cause of death, slight broncho-pneumonia.

It was desired to determine whether young ticks hatched from eggs laid by known infected parents, were capable of infecting a susceptible animal at their first feed.

Young ticks reared in the laboratory were allowed to feed upon this monkey. With the exception of eight young ticks which fed on Feb. 7, all the ticks used were hatched from eggs laid on Jan. 12 by one of the ticks which infected Ex. 157. The accompanying chart indicates the number of ticks fed, the temperature and the results of examination of the peripheral blood for spirochaetes. The animal was found to be infected on Feb. 9, and died, much exhausted, on Feb. 26. No autopsy was done.

If this monkey was infected, as we believe, by the tick bites, it may be assumed that ticks are not merely mechanical transmittors of the spirochaetes, but are alternative hosts in which developmental changes take place.*

Ex. 194: Cercopithecus, weight 2,265 grammes.

It was desired to determine whether young ticks hatched in the laboratory and fed on an animal infected with spirochaetes were capable of transmitting the parasite to a susceptible animal at any succeeding feed, twenty-four hours, at least, intervening between the infecting and transmitting feed. (This experiment was commenced before a successful result had been obtained in Ex. 192.)

Ticks were fed on Feb. 6, 7, 10, 23, March 2, 4, 16, and 19. The monkey was bitten 136 times in all. On March 21 spirochaetes were found for the first time in the blood. The temperature, previously rather irregular, mounted definitely. The animal became much prostrated, and lost weight rapidly. The temperature remained high; spirochaetes were constantly present in enormous numbers, and the animal died on the night of March 27 (weight 1,815 grammes).

We have, therefore, been able to infect, in all, eight animals with spirilla by the bites of ticks—seven monkeys and one rat.

To exclude the remote possibility of the parasite being introduced with dust into the wounds made by the ticks' bites and infection thus produced, on four different occasions 115 small incisions were made in the abdomen of a young Cercopithecus (weight 907 grammes), and earth taken from jars in which much-infected ticks were kept, and from tick-infested houses at Nyangwé was rubbed into the cuts. The animal never became infected.

The incubation period, that is, the interval elapsing between the bites of ticks able to infect at the time of biting† and the appearance of parasites in the peripheral blood, as a rule, is five days.

The Spirochaete.

When the parasites are numerous they can easily be seen in fresh preparations of blood as rapidly-moving spiral threads. When they are scanty perhaps the best method of demonstrating their presence is the searching, with a high power, of thick, dehaemoglobinised blood-films stained by some modification if Romanowsky's method or with a weak solution of carbol-fuchsin. Centrifugalizing the blood is not nearly so good for the demonstration of spirochaetes as for trypanosomes.

The parasites seen in all our cases have the same morphological characteristics. In preparations coloured by modifications of Romanowsky's stain (Leishman's or Stephens and Christopher's) (10) the spirochaetes are seen to stain unevenly, and to vary greatly in length. The results of the measurements of twenty spirochaetes in slides of blood taken from Case 8, for the most part on March 5, are as follows:—Largest 43 μ., smallest 13 μ., average of ten smallest 19 μ., average of ten largest 35 μ., Y-shaped, apparently divisional forms, and groupings of pairs, trios, and tetrads of spirochaete strongly resembling, in their arrangement, the multiplying indifferent

* October 17, 1905. This experiment has since been, twice, successfully repeated.

† Occasionally the bites of ticks, later shown to be infective, have repeatedly failed to infect a susceptible animal.

forms of *Spirochaete Ziemanni*—as figured by Schaudinn—(12)—are frequently seen in films taken from either patients or animals. With the time, instruments, and technique at our disposal, we have, to our regret, been so far unable to observe in animals and ticks developmental processes of this spirochaete similar to those which Schaudinn has demonstrated with *Spirochaete Ziemanni* in the owl and mosquito.

Distribution of the Human Tick in the Congo Free State and some Notes on its Binomics.

Livingstone says: "Before the Arabs came bugs were unknown" . . . one may know where these people have been by the absence or presence of these nasty vermin; the human tick, which infests all Arab and Suaheli houses, is to the Manuema unknown . . ." (2); and again, while at Nyangwé, "My new house is finished—a great comfort, for the other was foul and full of vermin; bugs (Tapasi or ticks) that follow wherever Arabs go made me miserable" (3). Our observations tend to confirm his assertion. Perhaps one of the reasons for which ticks are more often found in Arab than in native houses is that the Arabs make better, drier buildings, and live in permanent villages. Native huts are temporary affairs and a slight cause, one or two cases of sickness, is often enough to make a community leave their homes and build a village elsewhere.

In October 1903, a list of questions was sent for us by the Free State Government to its various districts. Information concerning the human tick was asked for. Since our arrival in the Congo and during our long journey up the river from Léopoldville to Kasongo we have constantly searched for the ticks. From the information thus collected we are enabled to construct the accompanying sketch map which shows the distribution of the human tick in the Congo Free State.

We have indicated every locality from which specimens have been received, or ticks are said, on reliable authority, to exist. Where it has been necessary to indicate places at which ticks are not known to exist squares, not circles, have been used to mark their position.

The main Arab routes into the Free State have been indicated diagrammatically by straight red lines. It must be understood that the Arabs overran the southern part of the Free State before reaching the Upper Congo at Nyangwé. Maniéma then became their stronghold, from which expeditions were sent out. They never penetrated far to the west of the Congo. The Free State prevented them from following the Congo further down than its confluence with the Aruwimi. Their progress to the north was checked, near Avakubi, and to the north-east, near Béni, by powerful native tribes. They were practically confined, in the Free State, to the territory now called the Oriental province.

Ticks seem to have come into the Free State by two routes; from the East Coast, with the Arabs, into the Oriental province, and into the Cataract region, with traders, from the Portuguese territory to the south, where ticks have existed since Livingstone's time, at least.

The rivers are the present highways. Old and present caravan routes used by Europeans, along which ticks are known to occur, are indicated by dotted lines. A glance at the map shows that ticks are found, particularly, along much travelled roads. How easily they may be carried in even a European's luggage is well shown by our experience on leaving Nyangwé, where we were well lodged in well-built houses. Although the ticks are plentiful in many of the Arabised villages along the Congo between Kasongo and Ponthierville, they are quite unknown in the native villages an hour's walk inland. The rest-houses for native travellers are always the most infested. At Kumba, Lokandu, and Ukungwa, the points furthest down the river at which we caught ticks, the rest-houses alone seemed to be infested. At Kumba the native paddlers of the town said they did not recognise, and seemed to have no fear of, the tick. Dr. Christy, in Uganda (11) and the Rev. W. Holman Bentley in the Lower Congo have also found that rest-houses are particularly liable to be infested.

Mr. Bentley believes that human ticks were a chief cause of the great mortality which existed among the Lower Congo porters in the old days when the whole commerce of the Free State was carried over the caravan route between Matadi and Léopoldville.

Names given to the ticks in various localities are as follow:—In the neighbourhood of Léopoldville *Bifundikala*, and, by the Batéké, *Bimpusi*. It exists along the Kwango river, at least between Popokabaka and Francis Joseph Falls, among the Basumbo and Bayaka people. At Popokabaka it is called *Mouyata*. The common name throughout the eastern part of the State is *Kimputu*. Very occasionally an educated man uses the Suaheli word *Papasi*.

In infected houses the ticks are found in the dust and cracks of mud-floors, particularly in dry places near the hearth, in bed-platforms, or immediately inside the door-sill, just where the natives are accustomed to sit down. They may hide themselves in the cracks and crevices of mud or grass walls, and even in the thatched roofs.

When ticks are disturbed they often curl up their legs as if dead. So lifeless do they seem that one might easily be deceived, especially since they sometimes remain motionless for hours.

Ticks can crawl rather quickly. In sand the body of a large one leaves a smooth, central furrow, with the sharp, crab-like tracks of the claws on either side. They seem to be largely nocturnal in their habits, and certainly do not feed quickly enough to get much blood from any but a sleeping person. A big female may remain, firmly fixed, feeding on a monkey for two or three hours before it finally drops off, as large as a cherry, distended and bloated with blood. Others may fall off and attempt to crawl away after half an hour's feeding. In feeding (Pl. 3, fig. 2), the tick first firmly fixes the forelegs, and then, depressing the capitulum, buries its mouth-parts in the host. The bite of even a small tick is painful. In monkeys, immediately after feeding, a small crust of sero-sanguinolent fluid forms at the site of the bite. Surrounding it is a roseola about two millimetres in width. Two hours later the central clot is surrounded by two concentric zones, each two millimetres in width; the first colourless, the second ecchymotic. Six hours later the

clot has become almost black, and is placed at the apex of a slight, colourless weal, bordered by an ecchymotic zone about a millimetre and a half in width.

It has been said that ticks bite most frequently between the toes, in the axilla, etc. Our experience indicates that they bite, indifferently, every part of the body.

Often, while still feeding, the whitish secretion of the Malphigian tubes is passed from the anus. At the same time clear fluid, in fairly large quantities may ooze intermittently from between the bases of the first and second pairs of legs. We have never seen fluid exude from any other situation than this; yet, in spite of careful search—no sections made—we have never been able to find any trace of an opening.

Spirochaetes have never been seen in this fluid.

In coitus the male lays hold of the posterior margin of the female, and, turning on his back, crawls forward, beneath the female, until the genital pores are in opposition. Pairs are often found and remain for hours *in coitu*.

The productivity of the females is certainly increased by large feeding. The number of eggs laid at a time seems to vary greatly. Dissection shows that only a few eggs are mature at one time: ovipositing therefore goes on slowly. On two occasions ticks have been seen to be ovipositing during three days. In each instance only seventy eggs were laid. Our ticks were frequently disturbed, and for this reason, perhaps, the eggs have been usually found in small groups of ten or twenty. Females kept apart for observation have, however, been found to lay from ten to twenty eggs at intervals of from one to two weeks. The largest number of eggs laid at one time was 139. The females have sometimes, but by no means always, died while, or after, ovipositing.

The eggs adhere to one another as they are laid, and look, under a low power, not unlike bunches of glistening, golden-brown grapes. Their covering is soft, smooth, and highly refractile. Their shape is slightly ovoid, the average dimensions being 881.172 by 776.376 μ. The largest egg measures 959.4 by 841.32 μ., and the smallest 767.52 by 664.2 μ. In sandy soil the eggs may be deposited either upon or beneath the surface. The average temperature and the average humidity for the three months during which we have had ticks under observation have been as follows:—

	Temperature		Humidity Percentages of Saturation		
	Maximum	Minimum	6 o'clock	11.45 o'clock	12.45 o'clock
1904 December	29·6° C	19·7° C	94	76·1	76
1905 January...	32·3° C	19·5° C	95	71·5	74
February	29·9° C	20·1° C	94	72·1	77

Under these conditions eggs take but twenty days to hatch; the shortest period noted was eighteen, and the longest twenty-three days. Occasionally, for no apparent reason, a quarter, or even more, of a batch of eggs has failed to hatch.

In about seven days' time the egg becomes more ovoid, and, with a low power (a 3 objective ; No. 2 ocular ; Zeiss, stereoscopic), the hexapod larva can be seen to be forming within the translucent shell (Pl. 1, fig. 2). At about the thirteenth day the egg-shell splits, nearly always posteriorly, in the sagittal plane. It measures at this moment 1.03 by 9mm. A hexapod larva (Pl. 1, fig. 4), which moves its legs but cannot crawl, may now be extracted from the shell. Normally the egg-case and its contents become batter, and the larva's skin wrinkled Pl. 1, fig. 7). Air enters beneath the egg-shell and the larval skin so that the young tick and his coverings form a dull, white disc. If the egg-shell be removed, it can be seen that the tick is about to moult. Its tegument has split anteriorly, sometimes laterally, in the usual line of ecdysis at the junction of the dorsal and ventral surfaces ; and curled up within the hexapod larval skin can be seen the eight-legged nymph. When ecdysis finally takes place, the nymph throws off egg-shell and larval cast together. It usually emerges by getting out one of its hind legs first, and then, often turning around inside its casings, dragging its body out after. The measurements of ten nymphs taken just after leaving the shell were, average 1.02 by 0.87mm., the largest 1.10 by 0.96mm., the smallest 0.98 by 0.8mm.

The newly-hatched nymph (Pl. 1, fig. 8) has no genital pore, and the adult position of the stigmen is indicated by a small white spot and pit. Its capitulum is partially retractile, and is visible from above. Their bodies, under a low power, are translucent. Freshly-hatched nymphs usually do not feed at once. As a rule only three or four days later do they readily suck blood. From observations extending over two months, based on ticks hatched in the laboratory from infected parents, but fed upon uninfected animals, it seems that if food is plentiful a young tick grows rapidly. The intervals between the moults seem to vary very considerably ; but in the two months after it leaves the shell the tick may moult three times and come to measure, roughly, 5 by 2.5mm. If food is lacking, growth is much less rapid.

A full-grown female, filled with eggs and blood, may measure 12 by 10 by 7mm. After the nymphs first moult the stigmen is easily distinguishable, but the genital pore is still absent. The measurement of twenty young ticks at this stage gave, average 1.47 by 1.22mm., largest 1.92 by 1.37mm., the smallest 1.34 by 1.07mm. It is only after the second moult that the sexual pore is seen as a very slight depression in the middle line of the ventral surface on a level with the bases of the first pair of legs. Cleavage of the skin for ecdysis always takes place along the line indicated in the diagram (Pl. 3, fig. 3). The split usually commences anteriorly. The feet and ventral surfaces are first freed, then the dorsum, and the perfect cast is left. Either dorsal or ventral half of the moult may, however, remain attached after the other moiety has been thrown off.

It is impossible to say how long the ticks live, or for how long they remain infective. Ticks caught on October 24, and kept without food, are still alive, and have freely reproduced. Ticks caught before November 23, still alive (March 28, 1905), and have freely reproduced. Ticks caught before November 23, at Nywangé, and kept without food, were able to infect a monkey with spirochaetes $1\frac{3}{4}$ months later.

Ticks are not without natural enemies. Rats eat adults with avidity, and ants carry off young ones and eggs. We have lost ticks in both ways. On one occasion over two hundred young ticks were carried off in a single night by small ants.

We add the following scanty working notes on the anatomy of *Ornithodoros*, and on the presence of spirochaetes in its organs, in the hope that they may be useful to others interested in this subject.

There is a most curious piece of anatomy in the adult tick which, for want of a better name, we have termed the (?) "pulmonary sac." Just above the base of the proboscis is a slit which communicates with a wide membranous tube ending in a paired body, or, rather, a closed sac, whose walls are at first thin, near its attachment to the tube, and distally are thickened and thrown into folds. The accompanying diagram (Pl. 4, fig. 1) illustrates this point.

It is curious that we have never seen these ticks excrete dark-coloured faecal matter. It is always white-coloured secretion of the malpighian tubules which is passed by the anus. This fact may, in some part, be explained by the exceeding fineness of the tube running from the under surface of the "stomach" to the central cul-de-sac of the cloaca. The diagram (Pl. 4, fig. 2) is very schematic, but it will serve to illustrate the course of the alimentary tract.

The position and rough structure of the salivary glands are indicated in a diagram (Pl. 4, fig. 3). The appearance of the salivary duct is very characteristic.

A batch of ticks caught at Nyangwé (November 23rd, 1904) were fed upon one of our infected monkeys. They were kept without further feeding, and one was dissected every day or two. Living spirochaetes were found in their stomachs and malpighian tubules up to five weeks after their feed on a known, infected animal. (It must be remembered that these ticks were naturally-infected adults.) Occasionally very many more spirochaetes were found in the tubules than in the stomach. In some preparations of stomach or malpighian tubules no parasites were at first seen; but if a little human serum, taken from one who had never had tick fever, were added, in from 8 to 24 hours the preparations became fairly crowded with spirochaetes.

As we have noted on page 13, we have so far been unable to follow out in these ticks Schaudinn's masterly observations. We have also been, up to the present, unable to find in the bone-marrow, spleen juice, or blood of monkeys or human beings infected with these spirochaetes forms resembling the large macro- or microgametes, described by Schaudinn in his work.

From the facts here presented we conclude that:—

(1). Tick fever is clinically identical with relapsing fever, and has for pathogenic agent a spirochaete.

(2). The spirochaete is probably *Spirochaete Obermeieri*.

(3). The tick, *Ornithodoros moubata*, can transmit the spirochaete from animal to animal.

(4). The transmission is not merely mechanical, but some developmental process is carried on in the tick.

(5). A considerable degree of immunity or tolerance to the spirochaete can probably be acquired.

* March 28th, 1905. We have just seen two other European cases.

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ON THE EXTERNAL ANATOMY OF ORNITHODOROS MOUBATA

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ON THE EXTERNAL ANATOMY OF ORNITHODOROS MOUBATA (MURRAY)

BY

ROBERT NEWSTEAD, A.L.S., F.E.S., ETC.

(LECTURER ON ECONOMIC ENTOMOLOGY AND PARASITOLOGY)

(WITH TWO PLATES)

A consignment of the ticks referred to in the previous paper on the Human Tick Fever seen on the Upper Congo was forwarded from the Congo Expedition by Drs. Todd and Dutton. The ticks arrived at their destination on June 8th, after a journey of three months duration. The majority of the animals had died during transit, but seven examples were still living, and with them were found a number of fertile eggs, larvae in various stages of development and several active nymphs. Dr. H. E. Annett kindly handed the whole consignment over to me with the request that the species should be determined, and that if any further details could be added that they should be given in an appendix to the Report of the Congo Expedition. The material to hand afforded ample means for the study of the external anatomy of this species, and as the larva and nymph do not appear to have been described, or the characteristics of the adult female very clearly defined, it seemed desirable that as full an account as possible should be given in order that future investigators may have a more ready means of determining this highly important, but somewhat obscure, member of the Argasidæ.

Ornithodoros Moubata, Murray.

(Plates 1, 2).

Ornithodoros moubata, Murray, Economic Entomology. Aptera p. 182, No. 31, two figures, 1877.

Nec *Ornithodoros savignyi* (Audouin), Neumann, Mem. Soc. Zool. France, IX., p. 26, 1896 (*ubi. synon*).

Ornithodoros savignyi, var. *cœcus*, Neumann, *op. cit.* XIV., p. 256, 1901.

Ornithodoros moubata (Murray), Pocock, Thompson Yates, and Johnston Laborotories Report, Vol. V., Pt. I., p. 188, pl. XV. 1903.

Ornithodoros savignyi, var. *cœcus* (1), (Neumann), Lancaster, Journ. of Trop. Medicine, p. 124, 1905.

Adult female: Dorsum dusky-brown, with irregular confluent blotches, rarely lines, of dusky ochreous-yellow; in some examples the blotches were confined to the anterior region, in others they extended almost to the posterior margin; underside usually somewhat paler than the dorsum, abdominal region largely occupied by dull orange-ochreous blotches, which gradually merge into the darker ground colour. Legs and rostrum of a pale,

(1) Calman, who contributes the article, gives the name *caecum*, evidently in error.

translucent, ochreous-white. Sculpture of dorsum fairly constant, but less pronounced in a partly engorged female than in the old and shrivelled examples. Capitulum (Pl. 2, Fig. 3); hypostome not much wider at the base than at the anterior third, margin converging inwards, teeth in three bilateral rows; mandibles (Pl. 2, Fig. 4, 4a), when fully extended, reaching to the second articulation of the palpi, digit with a blunt terminal tooth, exterior apophysis with two widely separated teeth; *interior apophysis bidentate*; palpi tapering towards the extremity second joint much the longest, sides of all the segments highly chitinised. Derm cells (Fig. 5, 5a) large, irregularly ovate, with one or two extremely minute spines at or near the margin, each cell surrounded by a band of dark chitine, the latter character being much more pronounced in the region of the posterior margin. Stigmata (Fig. 6) lunular, peritreme finely pitted and strongly chitinised. Dermal secretion glands (Pl. 2, Fig. 7) irregular in outline and variable in size, the subcutaneous tubes attached to these organs are branched and appear like bundles of muscular fibre; there is a group between the base of the capitulum and coxa 1, another between coxa 1 and 2, and a third between coxa 3 and 4, there is also a long bilateral series commencing immediately posterior to the genital pore. Legs (Pl. 2, Figs. 8, 8a, 8b, 8c) normal. Margin of genital pore deeply crenulated.

Nymph (Pl. 1, Fig. 8): Colour, pale ochreous-brown, legs translucent white with, in certain lights, a faint, pale ochreous tinge. Form broadly ovate. Dorsum thickly studded with minute papillae, increasing in size posteriorly, forming a regular crenulated fringe at the margin. Sculpture of dorsum somewhat resembling that of the adult females. Capitulum (Pl. 2, Fig. 2) partly projecting beyond the margin. Hypostome with two bilateral rows of teeth, basal pair minute, the remaining teeth are large and equal in size; mandibles strong; digit blunt, terminal. Palpi broad, stout, third segment the shortest, apical segment about equal in length to the second, and bears two or three short spines at the tip, all the segments have a broad marginal band of dark chitine. Legs (Pl. 1, Figs. 9, 9a) sparsely spinose, rather long; leg i. (Fig. 9) of seven segments, Haller's organ present; legs ii.-iv. (Fig. 9a) of eight segments, with the femora more dilated outwardly than in leg i. Anal orifice placed a little posterior to the insertion of leg i. Vaginal orifice absent. Spiracles minute, placed opposite insertion of leg iii.

Larva (Pl. 1, Fig. 4) sub-circular; colour dull purplish-brown, varying somewhat in intensity according to the age of the individual; but at or near the period of ecdysis the cuticle becomes more opaque and greyish in tone. At this stage, immediately posterior to the anal orifice, is a comparatively large, subcutaneous arrow-shaped, or Y-shaped, patch of white granular matter, evidently secreted by the nymph, which at this period has reached an advanced stage of maturity. Segmentation of legs not clearly defined after maceration in potash; femoral area of each leg with three pairs of equidistant spines; there are also a few isolated spines on the regions of the other segments; terminal tarsal segment (Fig. 5) of each leg broadly obtuse; claws short, stout, and curved. Haller's organ placed immediately posterior to the terminal tarsal articulation. Capitulum (Pl. 2, Fig. 1) within the anterior margin in the earliest stage, but in more mature larvae it projects slightly beyond; base of rostrum on the dorsal area with two

rather long stout spines ; hypostome with two broad bilateral angular teeth, on the anterior third ; mandibles extending considerably beyond the apex of the hypostome. Palpi (Pl. 11, Fig. 1) broad and stout, extending beyond the fully-extended mandibles ; each of the second and third segments with one minute, and one long stout spine, there are also two or three minute spines at the apex of the terminal segments. Margin of abdominal area finely and regularly crenulated ; cephalo-thoracic area also crenulated, but more faintly so and less regular. Dermis (Pl. 1, Fig. 7) with fine wavy lines. Spiracles apparently absent.

Ovum (Pl. 1, Fig. 1): In addition to the "golden brown colour" described by the authors, an irregular faint whitish polygonal reticulation and interrupted radiating streaks showed through the cuticle in the freshly laid examples. For particulars regarding the development of the ova see Report ante p. 15 and also the notes on the habits of the species in this appendix.

When treated in a 10 per cent. solution of boiling potash (KOH) the eggs containing the mature larvae changed to a rather bright red colour, and instead of softening under the treatment became hard and opaque. It was found necessary, therefore, to express the fluids from the body before maceration in the potash ; otherwise it was found impossible to examine the structural details.

The adult female of this species may readily be distinguished from *O. savignyi*, not only by the absence of eyes, as has already been pointed out by Pocock (*l.c.*), but also by the presence of the *interior bidentate apophyses* and the apparently narrower base of the hypostome. The authors of the Report of the Congo Expedition (p. 15) have called attention to the singular fact "that a clear fluid, in fairly large quantity, may oose intermittently from between the bases of the first and second pair of legs." They were, however, unable to find "any trace of an opening." I find, on examination, that there are groups of glands in the situations indicated, and also a third group between the base of the capitulum and the first pair of legs, and in addition to these there is a long bilateral series immediately behind the vaginal opening. All the glands have subcutaneous tubes, which later are finely branched and, as already stated in the diagnosis, these organs have much the appearance of bundles of muscular fibres. I find also that a group of similar organs exist in *Ixodes hexagonus*, Leach, but in this species the subcutaneous tubes are comparatively short and unbranched, and they bear a very striking resemblance to the so-called tubular spinnerets found in the Lecanid group of the Coccidae.

It is important to note that the co-types (3) which were kindly identified by Pocock (*l.c.*) as *O. moubata*, are still in the collection of the School of Tropical Medicine. Some specimens of the latter were prepared for microscopical study, and were found on examination to be specifically identical with the specimens recently received from the Congo Expedition. There should be no doubt, therefore, as to the correct identity of the species. I have placed Neumann's var. *cœcus* as a doubtful synonym, as, unfortunately, I have not been able to consult Neumann's original description, but Pocock (*l.c.*) considers it to be the same as Murray's *O. moubata*. On the other hand, Ray Lancaster (*l.c.*) has quite recently (1905) identified some examples

of Ornithodoros from Angola, West Africa, as belonging to Neumann's var. *cœcus*.

Seeing that the specific characters of *O. moubata* are now found to be so markedly distinct from *O. savignyi*, there should be no difficulty in clearing up these somewhat important discrepancies by those who are in possession of the types.

The authors of the Report on the Congo Expedition are to be congratulated on the discovery of the life cycle of *Ornithodorus moubata*, which has not been hitherto observed. In many respects the habits of this species are not unlike those of *Argas persicus*, but so far as I have been able to ascertain the inert character of the larva of *O. moubata* is unique among the Ixodinae, in that it passes the whole of its life within the egg.

Three original drawings illustrative of the development of the larva and nymph accompanied Drs. Dutton and Todds' report, but they were so damaged in transit as to be useless for reproduction.

Experiments with the Ticks Imported from the Congo Expedition.

Experiment 1, June 19.—Thirteen nymphs were placed in a hollow bamboo, which was fastened to the axilla of a young Macaque monkey (*Macacus cynomologus*). The ends of the tube were securely corked, and a large section was cut away, providing the young ticks with ample means of access to the host. As a further means of precaution against admission of light, the tube was covered with a thick cloth. Under these conditions the ticks were allowed to remain on the host for a period of five hours. Result—nil. All the ticks refused to feed.

Experiment 2, June 19.—Five adult female ticks were put into a tube similar to that in which the nymphs were placed, and fastened to an adult Macaque, but as the ticks refused to feed they were subsequently removed and placed together on the monkey in the hollow below the knee of the left leg, and carefully covered with a chamois leather. One of the ticks almost immediately attached itself to the host; this took place at about 1.30 p.m., and the female left the host, fully engorged, at 3 p.m.

Observations on the Engorged Tick and Her Progeny.

The female became very restive after feeding, and made repeated attempts to escape from the bright sunlight. She was put into a Petri dish on a deep layer of dry sand, in which she rapidly buried herself, and was subsequently placed in a dry incubator at an even temperature of 29C. After laying the first batch of eggs, she was removed from the Petri dish and placed in a partly hollowed out banana and enclosed in a cardboard box. The animal immediately left the banana and took up a position in a broad groove, where she was partly concealed, in which position she remained fixed and inert until long after the third batch of eggs were laid. No attention was paid to the banana; indeed, she made a hasty retreat from the fruit and got as far away from it as the limits of her cage would permit. Two additional batches of eggs were laid, both lots being protected for a few days beneath the body of the parent, but both lots finally slipped from beneath her and fell, intact, to the bottom of the box. She defaecated three times during the period of egg laying, the white fluid like faeces, when dry, left a white deposit resembling French chalk in texture. The actual process

of egg laying was not observed. The length of the tick before feeding was 7mm.; after engorgement 11.50mm. The weight before feeding was .0270grms., after engorgement .2602grms., or an increase of about ten times her original weight. This was not a very marked increase, as compared with certain other species of female ticks, which are known to increase to about thirty times their original weight after engorgement. Batches of eggs were laid on the following dates—during the night :—

June 29.—First batch of 17 eggs. These were found in a little cluster at the bottom of the Petri dish.

July 2.—Second batch of 51 eggs laid. These were protected for a few days beneath the body of the parent.

July 10.—Third batch of 26 eggs laid. These were also protected by the parent for a few days.

Development of the First Batch of Eggs.

June 30.—First day—subcutaneous reticulation evident. Poles slightly cloudy.

June 31.—Second to fourth day—no change.

July 3.—Fifth day—subcutaneous reticulation more distinct.

July 5.—Sixth day—form decidedly flattened. Legs and capitulum of larva showing faintly through the cuticle of the egg.

July 6.—Seventh day—Form unchanged. Legs more evident.

July 7.—Eighth day—"Arrow-shaped, patch of secretion" formed *beneath* the cuticle of the larva, near anal orifice.

July 8.—Ninth day—egg shell split. Larva fully formed.

July 14.—Tenth to fifteenth day—cuticle of both egg and larva increasing in opacity, finally forming a dull white disc.

July 15.—Sixteenth day—nymphs hatching. Several examples were seen to escape from the larval exuviæ anteriorly.

Development of the Second Batch of Eggs.

Although these were laid four days later than the first batch, some of the nymphs appeared simultaneously with those of the first batch—i.e., on July 15.

Development of the Third Batch of Eggs.

These were laid twelve days after the first batch, or eight days after the second batch, and the first nymph appeared on July 18, just three days later than the earliest appearance of the nymphs from the first batch.

It should, however, be noted that the nymphs from all three batches were quite erratic in their appearance, as examples from each lot of eggs continued to emerge over a period of twelve days, the last appearing on July 27th.

EXPLANATION OF PLATE I.

Fig. 1.—Egg First day. x circa 40.

Fig. 2.—Egg at about the eighth day, showing the split cuticle, the sub-lying larva, and the "arrow shaped' sub-cutaneous patch of secretion (ventral). x circa 40.

Fig. 3.—Egg at about the tenth day, with the posterior portion of cuticle broken away (dorsal). x circa 40.

Fig 3a.— A similar example seen in profile. x circa 40.

Fig. 4.—Larva removed from the egg (ventral). x circa 40.

Fig. 5.—Tarsus of the larva. x 250.

Fig. 6.—Marginal fringe of the larva (a) and nymph (b) at the period of ecdysis. x 250.

Fig. 7.—Dermal markings of the larva. x 250.

Fig. 8.—Nymph (as seen in optical section after maceration in potash). x 25.

Fig. 9.—Leg i of the nymph. x 50.

Fig 9a.—Leg iv of the nymph. x 50

Fig. 10.—Posterior margin of nymph, showing character of large derm cells (stained preparation). x 250

EXPLANATION OF PLATE II.

Fig. 1.—Capitulum of larva x 250.

Fig. 2.—Capitulum of nymph. x 250.

Fig. 3.—Capitulum of adult female. x 75.

Figs. 4, 4a.—Mandibles of adult female, *ap*. inner bitentate apophyses. x 250.

Fig. 5.—Derm cells of adult female (stained preparation). x 200.

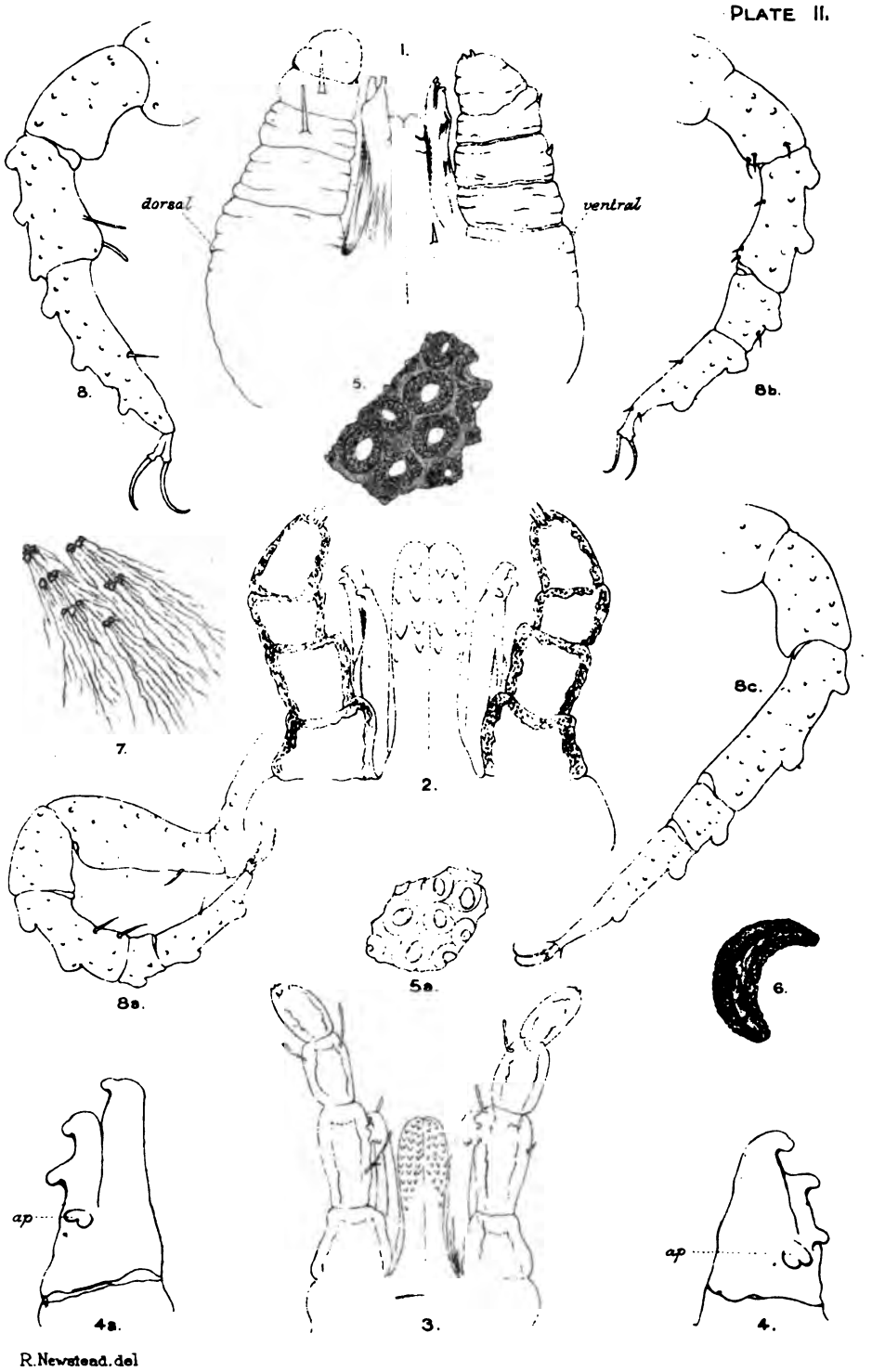
Fig. 5a.—Unstained preparation of same. x 200.

Fig. 6.—Stigmen of adult female, with its finely pitted peritreme. x 200.

Fig. 7.—Secretion glands of adult female. x 250.

Figs. 8, 8a, 8b, and 8c.—Leg i, ii, iii, and iv of adult female respectively. x circa 25.

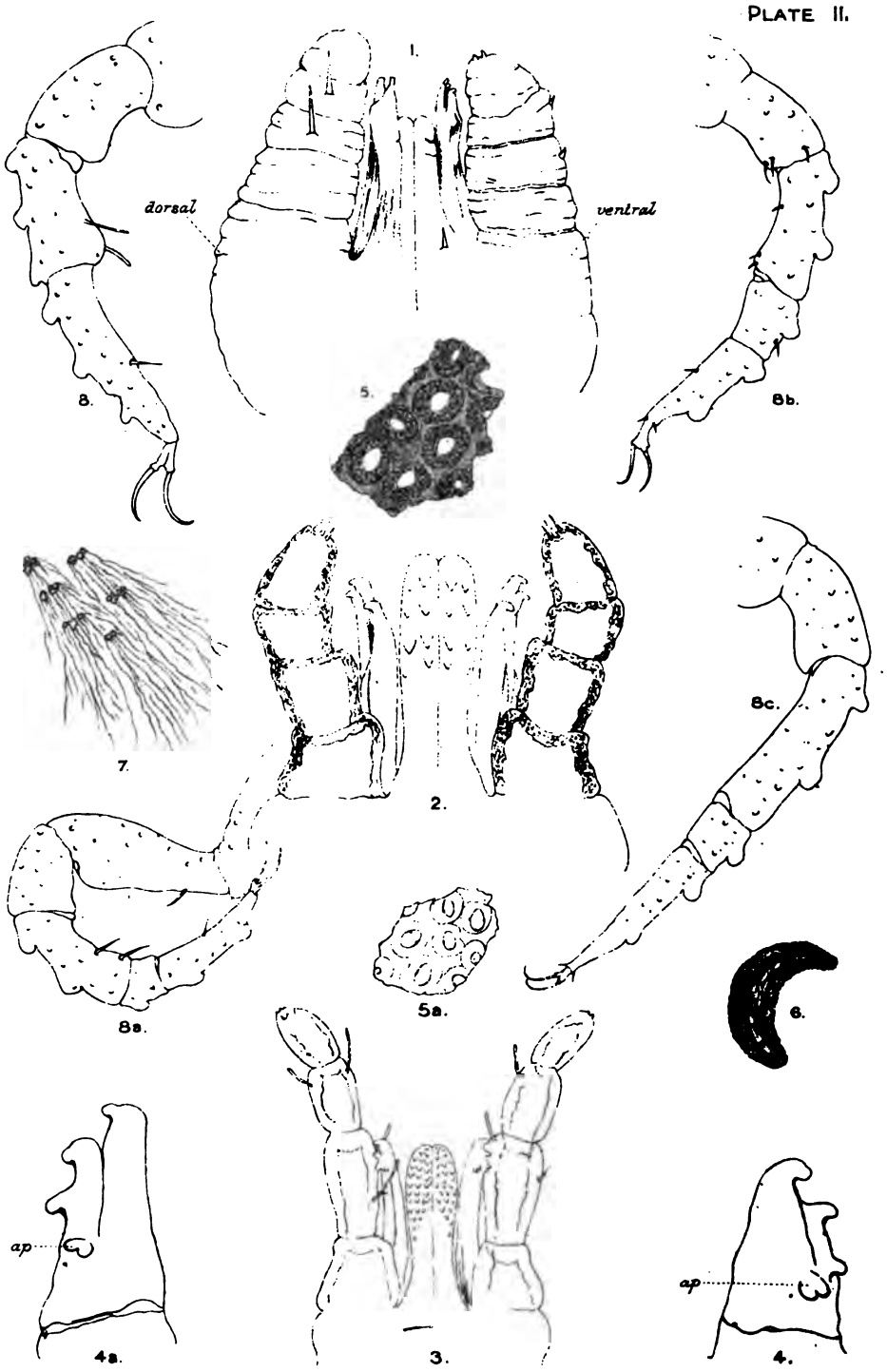
PLATE II.



R. Newstead. del

ORNITHODOROS MOUBATA.

PLATE II.



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ORNITHODOROS MOUBATA.

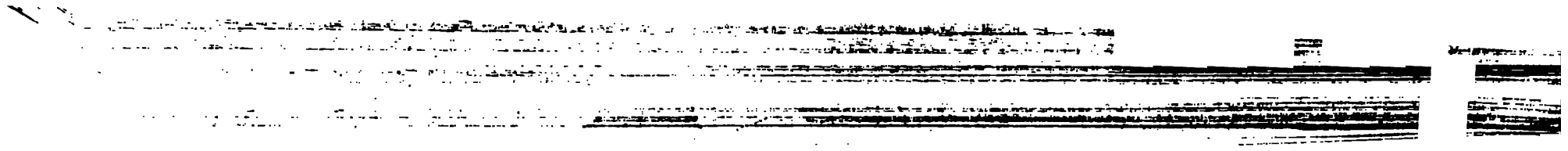

FIG 2. Ticks feeding on Monkey, Experiment 176

FIG. 1. To show enlarged spleen in Case IV

FIG. 3.
Line of ecdysis in adult tick

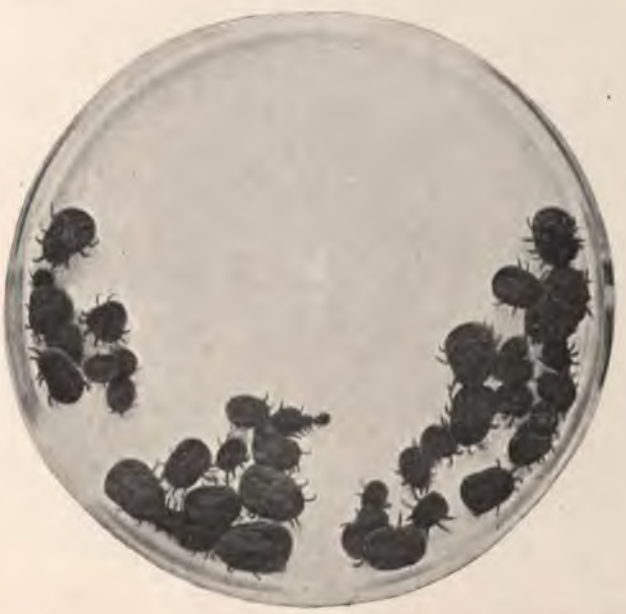

FIG. 4. Photograph from life of *Ornithodoros moubata* (slightly enlarged)

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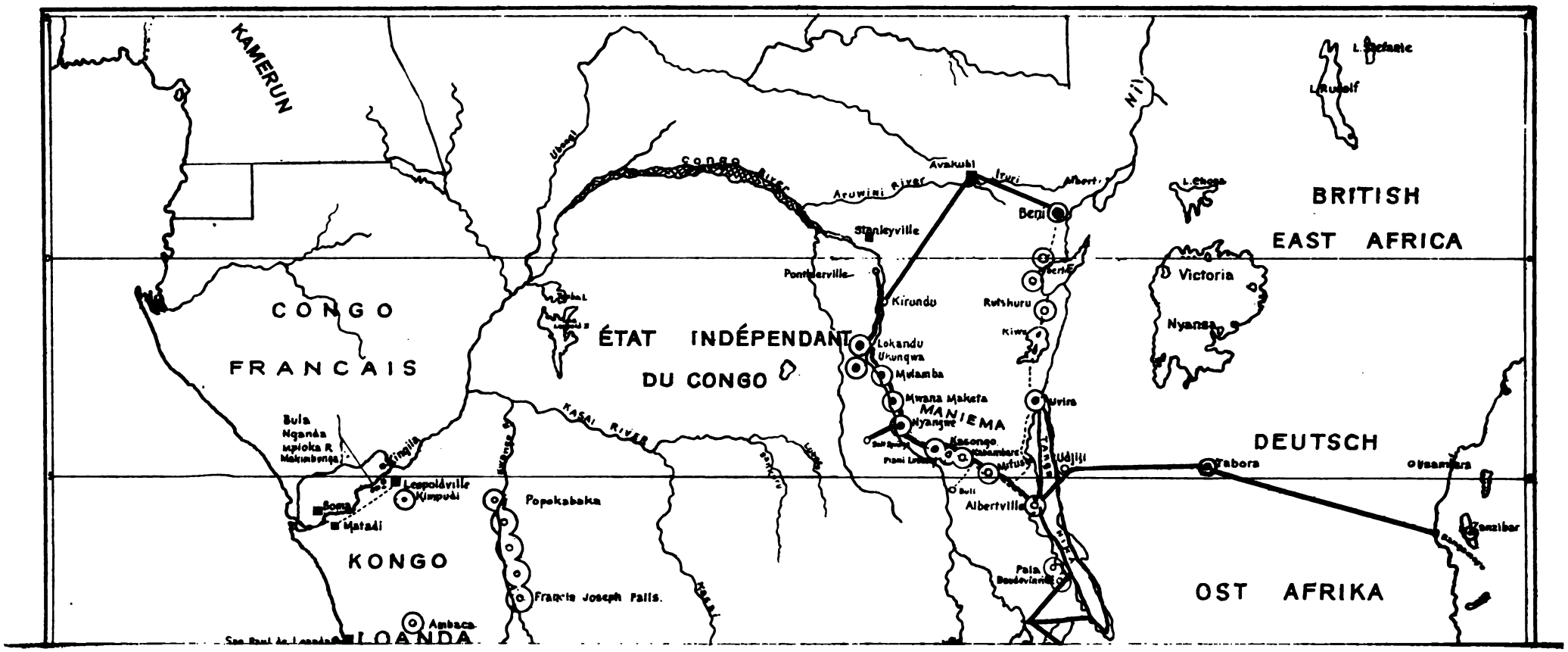
KAMERUN
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Congo River
Aruwimi River
Kasai River
Nile
Albert
Stanleyville
Kirundu
Lokandu
Ukunqwa
Mulamba
Mwana Makefa
Nyangwe
MANIEMA
Kasongo
Albertville
Bukama
Bukama
Kindu
Ponthierville
Avakubi
Irumu
Beni
Ruwenzori
Ruwenzori
Ruchuru
Kivu
Victoria
Nyanza
Mpala
Bukoba
Tabora
Zanzibar
Bula
Nganda
Mpioka R.
Matadi
Boma
Leopoldville
Kimpuki
Popokabaka
Francis Joseph Falls.
Ambaca
Luluabourg
Lubefu R.
Lomami R.
Lualaba R.
Ubangi
Uele
Semliki
Albert Edward
L. Tanganyika
L. Rudolf
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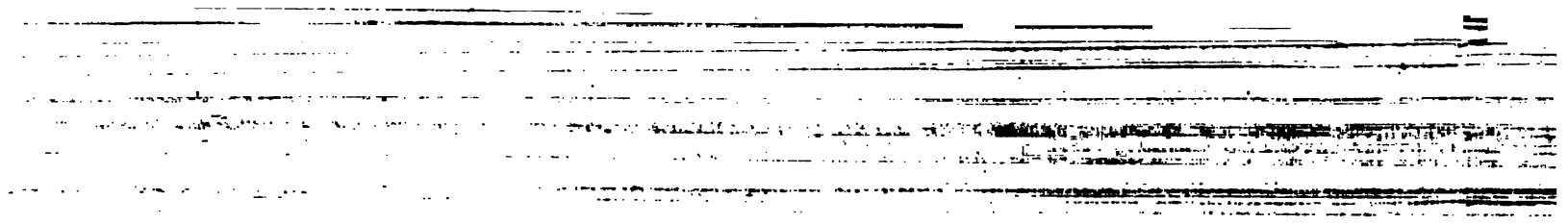
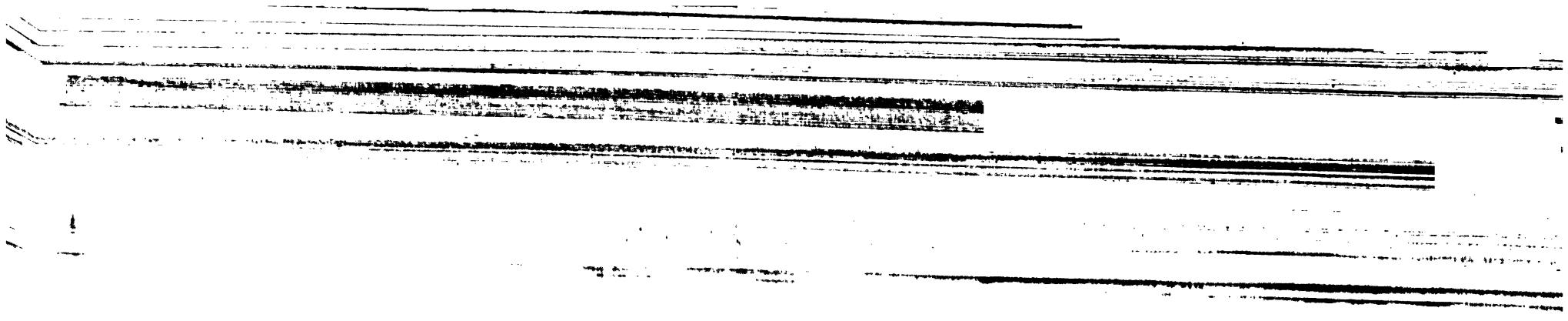


ON THE HABITS OF THE MARINE MOSQUITO

(*ACARTOMYIA ZAMMITII*, THEOBALD)



Acartomyia larvae are not found in pools which are connected with the sea itself, for, then, the water does not contain a sufficient degree of concentration of the salt. The water need not necessarily be clean, for we found *Acartomyia* in a pool on the rocks at Beyrut, which also contained old boots, tins, cabbages, and refuse of all sorts, and which, in some places, was six feet deep. The pools at Malta, however, are very clean, and only contain sea-weed.

The larvae do not seem to object to direct sunlight, for, in many of these pools, there is no shade, and these swarm with these mosquitoes. After the imago has hatched out from the pupa, the pupa-case floats for twenty-four hours; the female mosquitoes sometime utilize these floating pupa-cases to stand on when laying their eggs. At the end of about twenty-four hours the pupa-case sinks to a certain depth, where it is eaten by the young generation of larvae, so that the water is kept clean by the larvae themselves, unless extraneous decomposing matter is thrown into it.

The process of hatching takes about half-an-hour; the pupa becomes stationary at the surface of the water; then the back of its thorax splits transversely, and the head with the antennae and proboscis appear, followed by the two fore legs which are thrust out through the openings and rest on the surface of the water, their points making minute depressions on the surface of the water, which are very pretty to observe; the upper part of the thorax of the imago then appears, and the second pair of legs are placed upon the water behind the first. The upper part of the wings can then be seen, and when these are first delivered are folded together like a pair of scissors.

It is in this stage that the insect is most readily drowned, for the birth of the hind pair of legs and the abdomen takes by far the longest, but while this is going on the wings are opened to prevent wetting, and the half-hatched imago balances itself with them, while the hind pair of legs are slowly drawn out of the pupa-case.

When completely free of the pupa-case the imago rests for about ten minutes on the empty pupa-skin and is blown about by the wind; at first it is feeble, but it is soon able to fly away.

Only the fertilized females feed on blood.

We have tried scores of times to get the unfertilized females to bite, but have always failed.

The fertilized females can generally be recognized by the shape of the lower part of the abdomen, which is much fuller owing to the presence of the maturing eggs.

Coitus does not necessarily take place on the wing, for I have observed it occur when the female is resting on the edge of the glass jar or sitting on the rock; the male clasping the female when she is resting. When the female is waiting for the male she waves her hind legs over her back in a disconsolate sort of way.

Coitus may take place from one hour to twelve hours after hatching, and the fertilized females are ready to feed immediately after when the weather is warm.

Acartomyia zammitii will feed in the daytime as well as at night and even by strong artificial light.

I have watched the process of feeding carefully.

When one's hand is placed at the mouth of the jar containing a fertilized female, the mosquito, after one or two preliminary hops, settles on the skin. It then starts to clean its proboscis with its fore legs ; and then moves about apparently searching for a favourable site for its puncture. The proboscis is pushed into the skin quite slowly and sometimes is quickly withdrawn, as if the place chosen had been found unsuitable and another place sought. The process of filling usually takes about a minute, this differing from the biting fly (*Stomoxys*) found out here which fills itself in a few seconds.

I have noticed that only the tip of the proboscis pierces the skin, the proboscis being bent in the middle, the antennae being bent over the head while the animal is feeding.

We have only kept this mosquito alive for eight days in captivity, for they always have either died while laying their eggs, owing, we think, to the vibration of ship's dynamos, for we have been unable to work on shore.

I have caught one in a ship, however, which has not been anchored near the shore for two months ; and have also caught them in the winter. The larvae are first found in April and disappear from the pools in October.

Up to the present we have found *Acartomyia* at the following places :— Marmarice in Asia Minor, Beyrut, Port Said, Malta, Ajjaccio in Corsica, Algiers, Gibraltar (last year only) ; Algeceiras at neap tides only as the pools were washed out at spring tides.

Of the collection I am sending you, those numbered 1, 2, 3, were caught by me in houses in Port Said, where they were breeding in salt cesspools ; two of these contain blood.

The flask I am sending you contains some eggs of *Acartomyia* laid June 8 ; the mosquito was drowned while laying them, and is also in the flask. This mosquito had bitten a Maltese fever patient, and then for five days three non-immunes.

The water has evaporated and shows the salt crystalized on the bottom of the flask, for I placed some of the water in the flask from which the mosquito herself was hatched.

I think there is no doubt that Malta fever is conveyed by either *Acartomyia* or by a biting fly (*Stomoxys calcitrans*) which is very prevalent out here.

H.M.S. 'LANCASTER'
MEDITERRANEAN FLEET
September 14th, 1905

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